Soilborne Plant Diseases

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Proceedings of a Symposium on Soilborne Plant Diseases at Taiwan Agricultural Research Institute 15-16 November, 1984

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PREFACE

Soilborne plant diseases have attracted much research attention in the last ten years in Taiwan. The inspiration has come in part from realization of the magnitude of damage that a soilborne disease can do to a crop of economic importance. The Fusarium wilt of banana caused by a new race of *Fusarium oxysporum* f. sp. *cubense* is a case in point. Previously unknown to local banana growers, the disease was first found in 1967. It spread rapidly in subsequent years to produce devastating effects on commercial varieties of the Cavendish bananas which had been considered wilt resistant. No satisfactory control measures have yet been worked out for this destructive disease of soilborne nature. Justifications for placing emphasis on soilborne disease research also stem from some recent evidence that the incidence of crop diseases due to soil-inhibiting fungi and bacteria tend to increase noticeably. Among those contributing factors are the non-interrupted cultivation of same crops on the same land, heavy reliance on the use of chemical fertilizers, depletion of organic matter in the soil, and a gradual, though slight, increase in soil acidity in certain areas. All these developments combined intensify the soilborne disease problems that hamper crop production in Taiwan.

The Council of Agriculture has financed for the past three years a research program to study soilborne diseases of some major crops. Initially, the program embraced fifteen subjects to be undertaken by a number of plant pathologists. It was such designed that upon completion of pronounced misssions for the separate subjects, the participating researchers may either propose related subjects or leave the program. New subjects and new researchers are therefore entered from time to time, with the research remaining a team work.

It was the feeling of the Council of Agriculture and of the leaders of the program (S. K. Sun and Y. S. Lin) that after three-year implementation and upon entering a second phase, the program needs an objective, thorough review, preferably by well-experienced plant pathologists from abroad. Views expressed by the reviewers may form a basis for further strengthening or redirection, if necessary, of research emphasis. The 30th anniversary of the Plant Protection Society of the Republic of China held November 15-17, 1984 afforded such an opportunity. Instead of the usual reviewing panel, a symposium on soilborne plant diseases was organized as a conjunction activity to the Society's 30th anniversary. The Council of Agriculture, the Taiwan Agricultural Research Institute, the Plant Protection Society, Food and Fertilizer Technology Center, and Taiwan Provincial Fruit Marketing Cooperation co-sponsored the symposium. Among the participants were plant pathologists invited from abroad and local researchers teamed up under the said program.

We take this opportunity to express great appreciation to Drs. John L. Lockwood, R. James Cook, Tadao Ui, Tsuneo Watanabe, and W. H. Ko for their participation in the symposium and particularly for their highly inspiring, far-sighted views and unreserved appraisals of the research activities that the above program has focused.

The publication of the symposium's proceedings is made possible by a grant from the Council of Agriculture. All papers contained in this volume also appear in the Plant Protection Bulletin (an official journal of the Plant Protection Society of the Republic of China) vol. 27 no. 3, issued September 1985.

Ren-Jong Chiu
Shou-Kung Sun
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Mechanisms of Control of Fusarium Wilt Diseases by Amendment of Soil with S-H Mixture

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摘　　要

孫守恭　黃振文 (1985) S-H 土壤添加物防治鐮胞菌萎凋病之機制 (國立中興大學植物病理學系)

本研究中所用之蘿蔔黃葉菌 RS-S-3 菌株及西瓜蔓割病菌 FnC-B 菌株，在添加S-H混合物之土中，均有分生胞子及厚膜胞子發芽率低及發芽管易於瓦解之特性，以此特性可以測知 S-H 添加物之抑病機制。在鹼性營養液中，S-H添加物抑制胞子發芽之效力高。S-H 添加物中之礦灰及蚵殼粉爲分解蘿蔔黃葉病菌發芽管及抑制厚膜胞子發芽之主要成分，而礦灰爲分解西瓜蔓割病菌發芽管之主要成分。在礦灰之五種成分中，氯化鈣及氯化鐵能抑制蘿蔔黃葉病菌之胞子發芽，而氯化鐵可瓦解西瓜蔓割病菌之發芽管，也抑制厚膜胞子之發芽。土中施用 S-H 添加物後，眞菌及放線菌之密度增加，但細菌無增減。S-H 添加物之抑制胞子發芽效果可被添加 Rose Bengal, PCNB 或鏈黴素而全部或部份消失。土中施用 S-H 添加物，可減少西瓜根圈中蔓割病菌 14 倍，也減少西瓜根部被蔓割病菌之纏化達二倍多。在有病菌之土中，S-H 添加物增加西瓜根重三倍，根數也增加六倍。在無病菌之土中，S-H 添加物增加西瓜根重二倍，增加根數 4.7 倍。由以上研究結果得知，S-H 添加物防治鐮胞菌萎凋病之機制有數項因子，包括無機鹽類可直接抑制病原菌，間接的增高土壤 pH 及土中微生物數量以抑制病原菌，也能增加寄主根數及根重以及增強寄主之抗病力。

ABSTRACT

Isolate RS-S-3 of *Fusarium oxysporum* f. sp. *raphani* and isolate FnC-B of *F. oxysporum* f. sp. *niveum* were selected for the study because of their low percentage germination of conidia and chlamydospores and high percentage lysis of germ tubes on soil amended with S-H mixture. S-H mixture was more effective in inhibiting spore germination at high pH than at low pH in nutrient solution. Mineral ash and oyster shell of the component of S-H mixture caused

high percentage of germ tube lysis and strong inhibition of spore germination of *F. oxysporum* f. sp. *raphani* in soil. For *F. oxysporum* f. sp. *niveum*, mineral ash was the only component of S-H mixture capable of inducing germ tube lysis in soil. Among the five major components of mineral ash tested, $CaCl_2$ and $FeCl_3$ were inhibitory of conidial and chlamydospore germination of *F. oxysporum* f. sp. *raphani* and $FeCl_3$ was stimulatory to germ tube lysis and inhibitory to chlamydospore germination of *F. oxysporum* f. sp. *niveum* in soil.

Amendment of soil with S-H mixture increased the population of fungi and actinomycetes but not bacteria. Germination inhibition in soil amended with S-H mixture was nullified completely or partially by addition of rose bengal, streptomycin or PCNB. Amendment of soil with S-H mixture decreased the rhizosphere population of *F. oxysporum* f. sp. *niveum* more than 14 times and reduced colonization of watermelon roots by the pathogen more than 2 times. In the presence of the pathogen, S-H mixture increased root weight about 3 times and root number 6 times, whereas in the absence of the pathogen, it increased root weight about 2 times and root number 4.7 times.

Results of the study suggest that control of Fusarium wilt diseases by amendment of soil with S-H mixture is the result of a multitude of factors including direct pathogen suppression by its inorganic components, indirect pathogen suppression by raising the soil pH and by increasing the microbial population with its organic and inorganic components and increases in root number of the host and host resistant to infection by the pathogen.

INTRODUCTION

Although the possibility of using soil amendments to reduce soilborne diseases has been studied by many researchers from different countries, control of avocado root root rot caused by *Phytophthora cinnamomi* by annual incorporation of organic matter into the orchards in Australia appears to be the only case of successful application of this method in field scale[2]. Soil amendment has been a routine practice of farmers in the mainland China for hundreds or thousands of years mainly as a source of fertilizers. Recently it has been suggested that such practice might be the reason for the general absence of important root diseases on agricultural crops in China[2]. Soil amendment has been considered by many plant pathologists to be a promising method for controlling soilborne diseases in the future.

A soil amendment called S-H mixture consisting mainly of agricultural and industrial wastes has been developed recently for successful control of radish yellows[13] and watermelon wilt[5,16] in both greenhouse and field trials. We report here the possible mechanisms by which the S-H mixture suppresses the pathogens and the diseases caused by the pathogens in soil.

MATERIALS AND METHODS

Soil and S-H mixture

Loamy soil of a paddy field in Dali, Taichung was collected from depths

ranging from 0 to 15 cm after the surface litter was cleared. The soil was sieved through a 128-mesh screen, adjusted to about -5 bars moisture, and stored in closed plastic bags. It had the pH value of 5.3 and the composition of 35.2% sand, 38.8% silt, 26% clay and 1.4% organic matter. The S-H mixture (pH 8.0) consisted of 4.4% bagasse, 8.4% rice husks, 4.25% oyster shell powder, 8.25% urea, 1.01% KNO_3, 13.16% calcium superphosphate, and 60.5 % mineral ash[16]. Chemical composition of the mineral ash are 31% SiO_2, 44% CaO, 1.7% MgO, 18% Al_2O_3 and 1% FeO.

Selection of test organisms

To select isolates for bioassay, macroconidia of *Fusarium oxysporum* (Schl.) f. sp. *raphani* Kendrick & Snyder (isolate RS-0) and *Fusarium oxysporum* (Schl.) f. sp. *niveum* (E. F. Smith) Snyder & Hansen (isolate FnC-0) were obtained by growing the fungi on potato dextrose agar (PDA) under light (1900 Lux, 12 hr per day) at 24 C for 1 month, and placed on the surface of soil amended with 1% S-H mixture. After incubation for 16 hr at 24 C, conidia were scraped off the soil surface and suspended in 5 ml of distilled water in a petri dish. Non-germinated conidia were transferred to water agar and further incubated. After 16 hr, germinated conidia were each transferred to a PDA slant.

Germination and lysis of fungi

Macroconidia and chlamydospores of isolates of *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *niveum* were obtained by growing the fungi on PDA under light at 24 C for 15 and 65 days, respectively. Spores were suspended in 0.5 % glucose and 0.5 % asparagine solution for 1 hr before being placed on the surface of soil blocks as described by Ko and Ho[8]. After incubation, spores were stained with rose bengal [1% rose bengal, 5% phenol and 0.01% $CaCl_2$ in distilled water], scraped off the soil surface with a scalpel and destained on a glass slide with a drop of solution containing 5 N NaOH and 0.5 N NaCl[7]. Germination was determined after 16 hr incubation. For measuring length and estimating lysis of germ tubes, 20 hr incubation period was used.

Chlamydospore germination in liquid was tested in 100 ml of 0.5% glucose plus 0.5 % asparagine solution mixed with 1% S-H mixture in a 250 ml flask. Nutrient solution without S-H mixture was used as a control. The spore concentration used was about 10^5/ml. After incubation at 24 C for 12 hr, about 0.1 ml of the suspension from each flask was placed on a glass slide. Spores were stained with cotton blue and observed under microscope. At least 100 spores were examined for each treatment.

Determination of microbial population

Soil suspensions were prepared by grinding 10 g of soil with 90 ml of sterile distilled water. Serial dilutions (10^{-2}, 10^{-3} and 10^{-4} for fungi; 10^{-4}, 10^{-5} and 10^{-6} for actinomycetes; and 10^{-5}, 10^{-6} and 10^{-7} for bacteria) were plated on the following selective media: PDA plus 1000 ppm streptomycin for fungi, Chitin agar[11] for actinomycetes, tryptone agar[5] for bacteria, Nash PCNB agar[15] for *Fusarium* spp., and rose bengal-PNCB agar[12,14] for *Trichoderma* spp.

Pathogenicity test

About 2000 g of soil infested with 1.2×10^3 propagules/g. soil of *F. oxysporum*

f. sp. *raphani* in a 20-cm pot was planted with radish seeds (cultivar Meiwa). Three replicates were used and the disease incidence was recorded after 30 days. The same procedure was used to test the pathogenicity of *F. oxysporum* f. sp. *niveum* on watermelon (cultivar Hong Niang).

Host response

About 2000 g of artificially infested soil containing 1.2×10^3 propagules of *F. oxysporum* f. sp. *niveum* per g of soil was mixed with 1% (W/W) S-H mixture, placed in a 20-cm pot and planted with 2 watermelon seeds in the greenhouse. Non-amended infested soil was used as a control. After 30 days, population density of the pathogen in the rhizosphere soil and root colonization by the pathogen were determined with the Nash PCNB medium[15] using the method described by Banihashemi and deZeeuw[1]. Five replicates were used for each treatment.

Analysis of data

Unless otherwise stated three replicates were used for each treatment and all experiments were repeated at least once. Student's t-test was used when comparisons were made between two treatments and Duncan's multiple range range test was used when more than two treatments were compared.

RESULTS

Selection of test organisms

Among the four isolates of *F. oxysporum* f. sp. *raphani* tested, isolate RS-S-3 was the best organism for assaying the inhibitory effect of soil amended with S-H mixture (Table 1).

Table 1. Comparison of spore germination and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *raphani* on soils with and without amendment with 1% S-H mixture

Isolates	Conidial germination (%)		Chlamydospore germination (%)		Radish yellows (%)
	Amended soil	Non-amended soil	Amended soil	Non-amended soil	
RS-0	77 A[1)]	92 A	58 C	74 C	75 a[2)]
RS-S-B	48 B	91 A	41 C	59 C	45 b
RS-S-2	49 B	81 A	40 D	67 C	39 b
RS-S-3	36 B	82 A	43 D	74 C	82 a

1) Data followed by the same letter for each spore type of the same isolate are not significantly different at P=0.05 based on student's t-test.

2) Data followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

The isolate gave high percentage germination of conidia and chlamydospores on non-amended soil and low percentage germination on soil amended with S-H mixture. It was also strongly pathogenic to the host. Isolate FnC-B was the best among the four isolates of *F. oxysporum* f. sp. *niveum* for assaying the same effect (Table 2). Its germ tube showed only slight lysis on non-amended soil and

Table 2. Comparison of germ tube lysis, chlamydospore germination and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *niveum* on soils with and without amendment with 1% S-H mixture

Isolates	Germ tube lysis (%)		Chlamydospore germination (%)		Watermelon wilt (%)
	Amended soil	Non-amended soil	Amended soil	Non-amended soil	
FnC-0	15 A[1)]	2 A	48 D	81 C	89 a[2)]
FnC-S-1	5 A	4 A	50 C	65 C	72 b
FnC-S-2	9 A	3 A	20 C	31 C	66 b
FnC-B	31 B	6 A	65 D	93 C	94 a

1) Data followed by the same letter for germ tube lysis or chlamydospore germination of each isolate are not significantly different at P=0.05 based on student's t-test.
2) Data followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 3. Chlamydospore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) in nutrient solutions with or without 1% S-H mixture

Treatment	pH value	Germination (%)	
		F. oxysporum f. sp. *raphani*	*F. oxysporum* f. sp. *niveum*
S-H mixture[1)]	5.8	31 B[3)]	24 B
	8.0	12 C	4 C
None[2)]	5.8	81 A	74 A
	8.0	69 A	68 A

1) Original pH was 8.0.
2) Original pH was 5.8.
3) Data followed by the same letter in each column are not significantly different at P=0.05 according to Duncan's multiple range test.

extensive lysis on soil amended with S-H mixture. Chlamydospore germination of isolate FnC-B germinated completely on non-amended soil but only partially on amended soil. The fungus was also strongly pathogenic to its host. Isolate RS-S-3 of *F. oxysporum* f. sp. *raphani* and isolate FnC-B of *F. oxysporum* f. sp. *niveum* were, therefore, selected for used in this study.

Effect of S-H mixture on spore germination in nutrient solution

Chlamydospore germination of *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *niveum* were strongly suppressed in nutrient solution containing 1% S-H mixture (Table 3). S-H mixture was more effective in inhibiting spore germination at high pH than at low pH.

Effect soil amendment with S-H mixture and its components on the pathogens

Germination of *F. oxysporum* f. sp. *raphani* conidia and chlamydospores on soil was significantly decreased by amendment with S-H mixture, mineral ash, oyster shell, rice husks and urea, but was not affected by amendment with KNO_3, calcium superphosphate and bagasse (Fig. 1). S-H mixture was more effective in

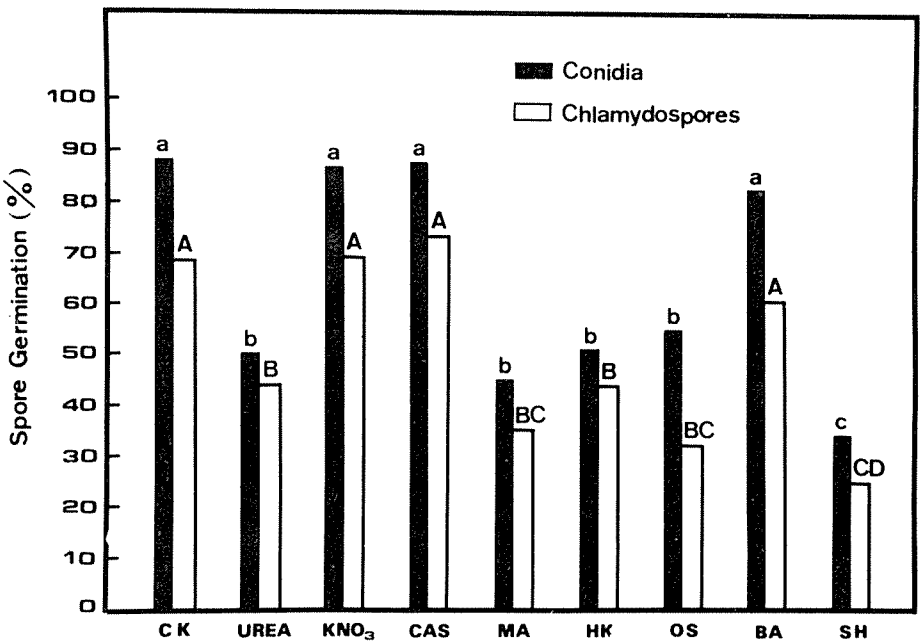

Fig. 1. Effect of amendments on spore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) on the soils. SH: S-H mixture, CAS: Calcium superphosphate, MA: Mineral ash, HK: Rice husks, OS: Oyster shell, BA: Bagasse. Data with the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

inhibiting spore germination than its individual component. Germ tube growth of the fungus on soil was strongly inhibited by amendment with S-H mixture, mineral ash, oyster shell, and urea and slightly inhibited by amendment with KNO_3, bagasse and rice husk, but was not affected by amendment with calcium superphosphate (Table 4). S-H mixture, mineral ash and oyster shell strongly enhanced germ tube lysis on soil. Amendment of soil with calcium superphosphate and bagasse also slightly increase lysis of germ tubes. Urea, KNO_3 and rice husk were not effective in inducing germ tube lysis. S-H mixture was more effective than any of its components in inducing germ tube lysis. Lysis of conidia and germ tubes of *F. oxysporum* f. sp. *niveum* on soil was enhanced by amendment with S-H mixture and mineral ash, but not with bagasse, rice husks, oyster shell, calcium superphosphate, KNO_3 and urea (Fig. 2).

Among the components of mineral ash only $CaCl_2$ and $FeCl_3$ significantly reduced germination of conidia and chlamydospores of *F. oxysproum* f. sp. *raphani* on soil (Table 5). $FeCl_3$ also enhanced lysis of germ tubes and

Table 4. Growth and lysis of germ tubes from chlamydospore of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) on soils amended with 1% of individual component of the S-H mixture

Treatment	Germ tube length (μm)	Germ tube lysis (%)
S-H mixture	56.2 A[1)]	52.0 A
Mineral ash	50.4 A	34.2 B
Oyster shell	65.0 A	26.4 B
Urea	63.4 A	5.6 D
KNO_3	98.0 C	4.2 D
Calcium-superphosphate	105.0 CD	14.0 C
Bagasse	87.2 BC	15.7 C
Rice husk	92.0 C	12.4 CD
Check (none)	124.1 D	6.2 D

1) Data followed by the same letter in each column are not different at P=0.05 according to Duncan's multiple range test.

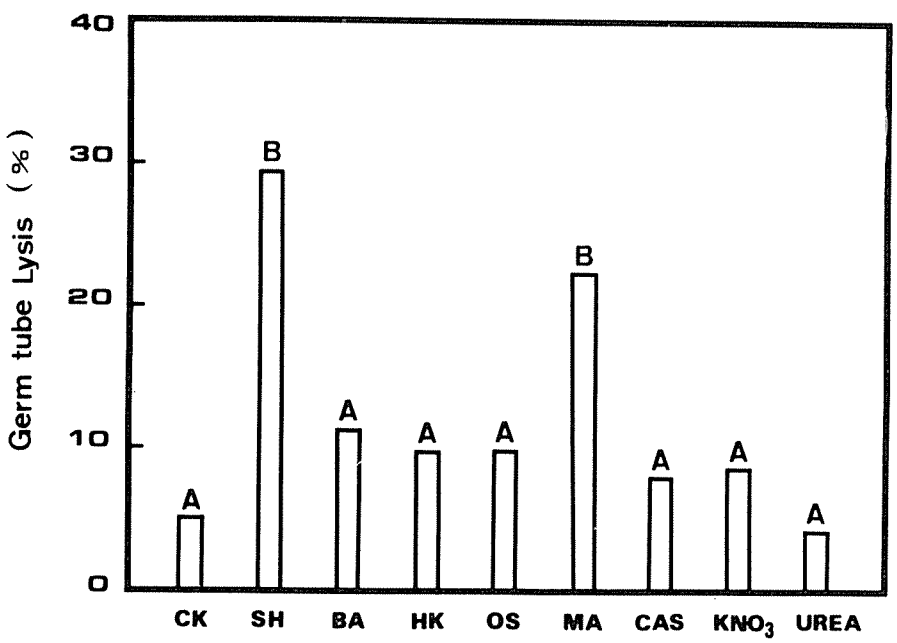

Fig. 2. Effect of amendments on germ tube lysis of *Fusarium oxysporum* f. sp. *niveum* (FnC-B) on the soils. SH: S-H mixture, CAS: Calcium superphosphate, MA: Mineral ash, HK: Rice husks, OS: Oyster shell, BA: Bagasse. Data with the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

Table 5. Spore germination and germ tube lysis of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) on soils amended with individual component of mineral ash

Treatment	Concentration (μg/g)	Soil pH	*F. oxysporum* f. sp. *raphani*		*F. oxysporum* f. sp. *niveum*	
			Conidial germination (%)	Chlamydospore germination (%)	Germ tube lysis (%)	Chlamydospore germination (%)
$AlCl_3$	150	4.9	83 A[1)]	79 A	5 A	87 A
$MgCl_2 \cdot 6H_2O$	200	5.3	92 A	87 A	8 A	84 A
H_2SiO_3	2000	5.4	81 A	84 A	2 A	91 A
$CaCl_2$	1000	5.3	54 B	57 B	9 A	75 A
$FeCl_3 \cdot 6H_2O$	100	5.0	58 B	61 B	18 B	56 B
Check(none)		5.3	79 A	77 A	4 A	85 A

1) Data followed by the same letter in each column are not different significantly (P=0.05) according to Duncan's multiple range test.

Table 6. Comparison of microflora in soils with and without amendment with 1% S-H mixture

Microflora	10^3 propagules/g. soil	
	Amended soil[1)]	Non-amended soil
Fungi	17.0 A[2)]	0.67 B[2)]
Actinomycetes	1010.0 A	480.0 B
Bacteria	34200.0 A	36500.0 A
Fusarium spp.	2.0 A	1.95 A
Trichoderma spp.	7.3 A	1.48 B

1) Microbial population was determined 30 days after amendment.
2) Data followed by the same letter for each group of microorganisms are not significantly different at P=0.05 based on student's t-test.

decreased germination of chlamydospores of *F. oxysporum* f. sp. *niveum* on soil.

Effect of S-H mixture on microbial population in soil

Thirty days after amendment of soil with 1% S-H mixture, the population of fungi increased about 25 times and that of actinomycetes increased about 2 times (Table 6). The population of bacteria in soil was not significantly affected by S-H mixture. Amendment of soil with S-H mixture increased the population of *Trichoderma* spp. about 5 times but did not affect the population of *Fusarium* spp.

Effect of microbial inhibitors on pathogen suppression by soil amended with S-H mixture

Inhibition of chlamydospore germination of *F. oxysporum* f. sp. *raphani* on soil amended with 1% S-H mixture was completely nullified by rose bengal and partialy nullified by streptomycin and

Table 7. Chlamydospore germination of *Fusarium oxysporum* f. sp. *raphani* (RS-S-3) and *F. oxysporum* f. sp. *niveum* (FnC-B) on the S-H mixture-amended soil and non-amended soil treated with microbial inhibitors

Microbial inhibitor	Concentration (μg/g)	Chlamydospore germination (%)			
		F. oxysporum f. sp. *raphani*		*F. oxysporum* f. sp. *niveum*	
		Amended soil	Non-amended soil	Amended soil	Non-amended soil
Rose bengal	100	78.6 A[1)]	75.6 A	69.0 B	83.2 A
Streptomycin	3000	65.6 B	82.0 A	85.0 A	83.0 A
PCNB	1000	68.6 B	77.9 A	57.0 C	87.6 A
Control		46.7 C	82.5 A	50.4 C	79.3 A

1) Data followed by the same letter in each column are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 8. Effect of S-H mixture on rhizosphere population of pathogen, root colonization by pathogen and root growth of watermelon in soil infested with *Fusarium oxysporum* f. sp. *niveum* (FnC-B)

Treatment	Infested with pathogen				Without pathogen	
	Rhizosphere population of pathogen (propagules/g. soil)	Root colonization by pathogen (%)	Dry wt. of root (mg/plant)	No. of roots/plant	Dry wt. of root (mg/plant)	No. of roots /plant
S-H mixture	218	36.2	349	16.0	593	21.0
None	3130	81.3	107	2.4	294	4.5

PCNB (Table 7). Germination of *F. oxysporum* f. sp. *niveum* on S-H mixture-amended soil was as high as non-amended soil when streptomycin was added. Rose bengal was slightly effective but PCNB was not effective in increasing germination of *F. oxysporum* f. sp. *niveum* on S-H mixture-amended soil.

Effect of S-H mixture on pathogen activity and growth of host in soil

Amendment of soil with S-H mixture decreased the rhizosphere population of *F. oxysporum* f. sp. *niveum* more than 14 times and reduced colonization of watermelon roots by the pathogen more than 2 times (Table 8). In the pathogen infested soil S-H mixture increased root weight and root number about 3 and 6 times, respectively. In the absence of the pathogen, S-H mixture increased root weight and root number about 2 and 4.7 times, respectively.

DISCUSSION

Amendment of soil with S-H mixture inhibited spore germination and induced germ tube lysis of both *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp.

niveum. Since S-H mixture in nutrient solution was inhibitory to spore germination of both fungi under sterile conditions, nonbiological factor apparently plays an important role in the pathogen suppression of S-H mixture-amended soil. Results show that mineral ash and oyster shell were the most important components of S-H mixture responsible for causing lysis of germ tubes and inhibition of spore germination of *F. oxysporum* f. sp. *raphani* in soil, although rice husks and urea may also contribute to the pathogen suppression to a limited extent. For *F. oxysporum* f. sp. *niveum*, mineral ash was the only component of S-H mixture capable of inducing germ tube lysis in soil.

Of the five major components of mineral ash, only $CaCl_2$ and $FeCl_3$ were inhibitory to conidial and chlamydospore germination of *F. oxysporum* f. sp. *raphani*, and only $FeCl_3$ was stimulatory to germ tube lysis and inhibitory to chlamydospore germination of *F. oxysporum* f. sp. *niveum* in soil. Oyster shell is rich in chitin and calcium. Its inhibitory effect in soil may be due to direct inhibition by calcium and indirect inhibition by chitin through stimulation of actinomycetes in soil[4]. Calcium has been shown to increase resistance of tomato to infection by *Fusarium oxysporum* f. sp. *lycopersici*[3].

Being alkaline in nature the S-H mixture tends to increase soil pH[16]. Since high pH soils disfavor the development of radish yellows caused by *F. oxysporum* f. sp. *raphani* and watermelon wilt caused by *F. oxysporum* f. sp. *niveum* [5,13], pH change may also contribute to the pathogen suppression in S-H mixture-amended soil although the extent of its contribution is still unknown.

Amendment of soil with S-H mixture also increased the populations of fungi and actinomycetes. It is considered possible that enhanced microbial activity may contribute to inhibition of sporangial germination[9] and lysis of germ tubes[10] in the S-H mixture-amended soil. The possibility is supported by the observation that addition of rose bengal, streptomycin or PCNB to soil amended with S-H mixture increased spore germination. Rose bengal and streptomycin are inhibitory to bacteria and actinomycetes but not to fungi[6], whereas PCNB is inhibitory to actinomycetes and certain fungi but not bacteria[4]. This also suggests that the microorganisms involved are not specific.

S-H mixture also has profound positive effect on the growth of the host. Watermelon growing on S-H mixture-amended soil produced about 4.7 times more roots than that growing in non-amended soil. This may account at least in part for the lower rhizosphere population of the pathogen and lower percentage of root colonization by the pathogen in S-H mixture-amended soil than that in non-amended soil.

Results of this study suggest that a multitude of factors are contributing to the control of Fusarium wilt diseases by amendment of soil with S-H mixture. S-H mixture may suppress the pathogen directly with its inorganic components and indirectly by raising the soil pH and by increasing the microbial populations with its organic and inorganic components. The calcium content of S-H mixture may increase the resistance of the host to infection by the pathogen. S-H mixture

may also provide nutrients for vigorous root growth of the host which in turn may also reduce the damage caused by pathogen infection.

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Natural Suppression of Soilborne Plant Diseases

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摘　　要

柯文雄（1985）抑制土壤傳染病害的自然方法　（美國夏威夷大學）

根據土壤樣品分析，*Pythium splendens* 的孢囊發芽率與土壤的 pH 值成負相關。高含量的鈣和高密度的微生物是抑病的原因。在已測定過的土壤樣品中，*Rhizoctonia solani* 的菌絲生長與土壤 pH 值成正相關。抑菌作用乃是由微生物的活動及高量的鋁離子所引起。一個由森林地所採集對 *Phytophthora capsici* 及其所引起的病害有抑制作用的土壤，以高壓蒸氣殺菌或在 500C 高溫處理十六小時後，這個土壤的抑菌作用仍舊存在。抑菌因子存在於土壤中的粘粒，坍粒及砂粒中，但不存在於水的抽出液中，由此可知 *P. capsici* 是被抑菌土中的無機顆粒所抑制。

ABSTRACT

There was an inverse relationship between percentage of germination of *Pythium splendens* sporangia and soil pH among the soil samples tested. A combination of high Ca content and high total microbial population was found to be responsible for inhibition of *P. splendens* in a suppressive soil.

There was a direct relationship between relative hyphal growth of *Rhizoctonia solani* and soil pH among the soil samples tested. Inhibition of *R. solani* in a suppressive soil was shown to be the result of microbial activity and high Al ion concentration.

A forest soil suppressive to *Phytophthora capsici* and the disease caused by the pathogen remained suppressive after autoclaving or ignition at 500°C for 16 hr. The inhibitory factors existed in the fractions of clay, silt and sand but not in the aqueous extract of suppressive soil, suggesting that inorganic soil particles are responsible for inhibition of *P. capsici* in this suppressive soil.

INTRODUCTION

Lower incidence of soil-borne disease in a given field compared with that of a nearby area has been observed frequently by growers with inquiring minds. However, the number of reports concerning such natural ways of controlling soilborne diseases so far has been very limited[1,2,9]. The facts that the fungistasis potential varies greatly among different soils[3] and that both volatile and non-volatile inhibitory substances exist in certain soils[4,11,12,13] strongly suggest that disease- and pathogen-suppressive soils may be relatively common in nature and may also exist in small islands in a conducive field. Both possibilities have been shown to be correct when a large number of soils were tested with a simple and rapid method developed to detect the inhibition of pathogens in a small amount of soil.[10] In 109 soil samples collected from the islands of Hawaii, Maui, Molokai and Kauai, about 40% were suppressive to *Pythium splendens* and 10% to the disease caused by this fungus[6]. For *Rhizoctonia solani*, about 14% of the 111 soil samples tested were suppressive to the pathogen and 5% to the disease caused by this fungus[5]. Among the five soil samples collected from a three-acre area of uniform soil texture at Lalamilo Experimental Farm, one was suppressive and four were conducive to *P. splendens*. In another experiment three out of 12 soil samples collected from a one-acre area of pasture at South Kohala were suppressive to *R. solani* and the other nine were conducive, although all were suppressive to *P. splendens*[10]. These results confirm the suggestion that suppressive soils may exist in small islands in a conducive field.

PYTHIUM SPLENDENS-SUPPRESSIVE SOIL

Sporangia of *P. splendens* suspended in cucumber root extract to overcome the general fungistasis were placed on the smooth surface of a soil block on a glass slide and stained with rose bengal after incubation at 24°C for 6 hr. Germination was counted directly on the soil surface using a vertical illumination microscope[10]. There was an inverse relationship between percentage of sporangial germination of *P. splendens* and soil pH when 31 soil samples collected on the island of Hawaii were analyzed. A similar relationship was obtained when 81 soil samples from the islands of Hawaii, Maui, Molokai and Kauai were analyzed. These results suggest that near neutral to alkaline soils in Hawaii tend to be more suppressive to *P. splendens* than acid soils[6].

A pasture soil in South Kohala which was highly suppressive to *P. splendens* and damping-off of cucumbr caused by the pathogen was selected for studying the mechanism of pathogen suppression. The suppressive soil remained suppressive when its pH was adjusted from the original 6.8 to 5.4 of the conducive soil, indicating that soil pH is not a contributing factor. Several lines of evidence suggest that non-specific microorganisms are involved in suppression of *P. splendens* in South Kohala soil[7]:

1. Inhibition of *P. splendens* by suppressive soil decreased with increasing depth of soil which was associated with decreasing populations of

bacteria, actinomycetes and fungi.

2. Sterilization by autoclaving or gamma-irradiation, or reduction of microbial population by steam treatment, completely eliminated the inhibitory effect of suppressive soil. Inhibition was restored to sterilzed suppressive soil by reinfestation with a suspension of either suppressive or conducive soil, or isolated bacteria, actinomycetes or fungi.
3. Inhibition was partially nullified by amendment of suppressive soil with rose bengal, streptomycin (both inhibitory to bacteria and actinomycetes) or benomyl (inhibitory to fungi).

Although microorganisms are associated with suppressiveness of South Kohala soil, the microbiological factor alone is insufficient to produce the inhibitory effect because of the following observations[3]:

1. Infestation of autoclaved conducive soil with suppressive soil suspension did not render its inhibitory.
2. Colonized gamma-irradiated conducive soil remaind conducive even though its total microbial population was higher than that in natural suppressive soil.
3. Adjustment of total microbial population in conducive soil to the same level as that in suppressive soil did not render it inhibitory.

These results suggest that a combination of biotic and abiotic factor are responsible for the suppressive effect of the South Kohala soil.

Since *P. splendens* suppression in Hawaii usually occurs in high pH soils some of which are known to contain high Ca content, pathogen suppression was compared with Ca content and total microbial population in suppressive and conducive soils. It was found that inhibition of *P. splendens* in suppressive soils was associated with high Ca content and high total microbial population. Amendment of conducive soil with $CaCO_3$, or increasing the microbial population in conducive soil by adding pure cultures of microorganisms or by amending the soil with alfalfa meal only slightly affect sporangial germination of *P. splendens*. However, when conducive soil was amended with $CaCO_3$ and microorganisms or $CaCO_3$ and alfalfa meal, germination of *P. splendens* decreased from 89% to 46% and 18%, respectively[8]. Stored soil and subsoil from South Kohala are high in Ca content and low in microbial populaton and are not inhibitory to *P. splendens*. Increasing the microbial population by adding water to stored soil or amending subsoil with alfalfa meal decreased the germination of *P. splendens* from 93% to 10% and 86% and 24%, respectively. The inhibition characteristics of artificially induced suppressive soil (conducive soil plus $CaCO_3$ and alfalfa meal) were similar to those of natural suppressive soil. It is therefore suggested that a combination of high Ca content and high total microbial population is responsible for inhibition of *P. splendens* in South Kohala soil[8].

RHIZOCTONIA SOLANI-SUPPRESSIVE SOIL

A polycarbonate membrane disc containing mycelia of *R. solani* was placed at one end of a soil block in a large plastic petri dish. Linear growth of

R. solani hyphae on the soil surface was recorded under a stereomicroscope every two days for four days[10]. Relative hyphal growth of *R. solani* on each soil was calculated as a percentage of hyphal growth on conducive Hilo soil. There was a direct relationship between relative hyphal growth of *R. solani* and the pH value of soil when 60 soil samples collected from the island of Hawaii were analyzed. A similar relationship was obtained when 111 soil samples collected from the islands of Hawaii, Maui, Molokai and Kauai were analyzed. These results suggest that acid soils in Hawaii tend to be more suppressive to *R. solani* than near neutral to alkaline soils[5]. The Onomea forest soil from the island of Hawaii, which was suppressive to *R. solani* and damping-off of mustard cabbage caused by the pathogen, was selected for studying the mechanism of inhibition.

When conducive soil was mixed with 25,50 or 75% of suppressive soil, relative growth of *R. solani* on conducive soil decreased with increasing amount of suppressive soil. However, growth inhibition in conducive soil did not increase during the incubation period, indicating that the suppression factor did not increase in the conducive soil; therefore, it is not transferable.

Inhibition of *R. solani* in suppressive soil is associated with microbial activity because autoclaving the soil eliminated the inhibitory effect and the inhibition was restored to autoclaved soil by reinfestation with soil microorganisms. Suspensions of both suppressive and conducive soils were capable of restoring inhibition to autoclaved suppressive soil but not autoclaved conducive soil, indicating that inhibition of *R. solani* in Onomea soil is the result of a combination of nonspecific microorganisms and an abiotic factor[5].

When the pH of suppressive soil was adjusted from the original 4.2 to 7.0, the relative hyphal growth of *R. solani* increased from 16% to 105%. The suppressiveness was not restored to the soil when its pH was readjusted to 4.2. Damping-off of mustard cabbage caused by *R. solani* increased from 26% to 100% when the pH of suppressive soil was adjusted from 4.2 to 7.0.

Extracts of suppressive soil but not conducive soil were inhibitory to hyphal growth of *R. solani*. The inhibitory effect also disappeared when the pH of the soil extract was adjusted from the original 4.2 to 7.0. Since germination of *Neurospora tetrasperma* was also inhibited in suppressive soil extract but not conducive soil extract and it required only 6 hr for each test rather than four days using the hyphal growth of *R. solani*, it was used as a bioassay in subsequent tests. Ascospores of *N. tetrasperma* germinated 33 to 41% in suppressive soil extracts and 93% in conducive soil extract. The Al ion concentration in the suppressive soil extracts ranged from 3 to 5.6 ppm, while that in the conducive soil extract was 1.6 ppm. Germination of *N. tetrasperma* ascospores in water containing 0,5 and 10 ppm $AlCl_3$ was 92,35 and 0%, respectively. These results suggest that Al ions may be an abiotic factor contributing to the inhibition of *R. solani* in Onomea soil[5].

PHYTOPHTHORA CAPSICI-SUPPRESSIVE SOIL

Sporangia of *Phytophthora* usually germinate indirectly by releasing zoospores in the absence of nutrients and directly by producing germ tubes in the presence of nutrients. Therefore, *P. capsici* sporangia were preincubated in 50% V-8 juice for 1 hr before being added to smooth surface of soil block to ensure uniform direct germination. A forest soil collected from the island of Hawaii was found to be strongly suppressive to sporangial germination of *P. capsici* and damping-off of tomato seedlings caused by the pathogen. Increasing the nutrient concentration in the sporangial suspension before addition to the soil did not increase sporangial germination, indicating that nutrients were not effective in overcoming the inhibitory effect of suppressive soil. When the pH of suppressive soil was adjusted from the original 4.2 to 7.0, sporangial germination of *P. capsici* increased from 3% to 35%. Adjusting the pH of coducive soil from the original 7.0 to 4.2 also decreased the germination from 92% to 30%. These results suggest that, although low pH is inhibitory to *P. capsici*, it alone cannot account for the strong inhibitory effect of the Hawaii soil against this pathogen [14].

Subsoils collected at depths of 30, 45, 60, and 75 cm from the suppressive area were also strongly inhibitory to sporangial germination of *P. capsici*. The suppressive soil was still inhibitory to *P. capsici* after autoclaving, indicating that living organisms were not responsible for the suppression. The inhibitory effect of the suppressive soil was not decreased after ignition at 500°C for 16 hr or treatment with H_2O_2 to remove organic matter. These results suggest that the inhibitory factor in the suppressive soil is inorganic and is heat stable.

To determine if the inhibitory factor is water soluble, 25 g of suppressive soil was shaken with 25 ml of water for 2 days[12]. The extract was not inhibitory to sporangial germination of *P. capsici*. When *P. capsici* sporangia were placed on a polycarbonate membrane (0.2 μm) on the soil, germination increased from 1% without membrane to 92% with membrane, suggesting that the inhibitory factor in the suppressive soil is not diffusible. The fractions of clay, silt and sand of the suppressive soil were all inhibitory to *P. capsici*. Results suggest that inorganic soil particles are responsible for the inhibition of sporangial germination of *P. capsici* in the suppressive soil[11].

DISCUSSION

Through extensive survey of pathogen-suppressive soils in nature in Hawaii, and intensive study of inhibition mechanisms of some of these suppressive soils, several important features concerning such natural ways of controlling soilborne diseases have been revealed:

1. Soils suppressive to different pathogens appear to exist commonly in nature.
2. Suppressive soil may cover the whole field. It may also be localized to form small patches in a conducive field.
3. Abiotic components of soil play an important role in pathogen inhibition in different suppressive soils.
4. Different pathogens may be inhibited by different mechanisms in the sup-

Table 1. Nature of soils suppressive to pathogens in Hawaii

	Soil suppressive to		
	Pythium splendens	*Rhizoctonia solani*	*Rhytophthora capsici*
1. Soil			
Classification	Ustollic Eutrandepts	Typic Hydrandepts	Typic Hydrandepts
Type	Very fine sandy loam	Silty clay loam	Silty clay loam
pH	6.8	4.2	4.2
2. Treatment			
Autoclaving	C[1)]	C	S
Nutrient amendment	C	N.T.[2)]	S
pH adjustment	S[1)]	C	M.S.[1)]
3. Mechanism			
Microorganisms	Nonspecific	Nonspecific	Not involved
Abiotic factor	Ca	Al^{3+}	Inorganic particles

1) S: Suppressive; M. S.: Moderately suppressive; C: Conducive.
2) N. T.: No test.

pressive soil (Table 1).

The general application of these findings regarding suppressive soils remains to be investigated in other parts of the world.

A suppressive soil like a sick plant is a general term. Description of symptoms of each sick plant is important to the distinction of one sick plant from the others, be it a wilt, blight, canker or mosaic, likewise description of inhibition characteristics of each suppressive soil is essential for its identification.

Studies of the mechanism(s) of suppression of a pathogen in a suppressive soil ultimately require experimentation whereby a conducive soil is made suppressive. Such experimentation may involve transfer of a specific organism or group of organisms, alteration of population of indigenous microorganisms, or adjustment of physical and chemical properties of soil or a certain combination of these. It is essential in such studies however that criteria be used to make certain that any suppressive soil created artificially operates by the same mechanism(s) operative in the same natural suppressive soil. There are many ways a plant may be diseased, similarly there are probably numerous ways a pathogen can be suppressed in the soil. Just as completion of Koch's postulates for establishing the pathogenicity requires the reproduction of the exact symptoms of a disease, so completion of the equivalent of a "Koch's postulates" for establishing the suppression mechanism(s) will require that the suppressiveness created is exactly the same as that operating naturally. This major step will depend on a careful comparison of the inhibition characteristics of natural and created suppressive soils.

Distinct inhibition characteristics of pathogen-suppressive soil may include:

1. Inhibition spectrum. Effect of a suppressive soil on different microorganisms could range from inhibitory to stimulatory[7].
2. Effect of pH adjustment. Certain suppressive soils partially or completely lost the inhibitory effect after adjustment of soil pH[2,5,14], while the inhibitory effect of others was not affected by pH change[7].
3. Effect of high-temperature treatment. Although most suppressive soils lost the suppressiveness after heat treatment[2,5,7], the inhibitory effect of some suppressive soils was not decreased by the same treatment[15,14].
4. Response to selective inhibitors. Different types of suppressive soils may respond differently to the same selective inhibitors[2,7]. Response of a suppressive soil to certain selective inhibitors also indicates the association of certain group(s) of microorganisms with the inhibitory effect of the suppressive soil.
5. Response to nutrient amendment. Nutrients may or may not counteract the inhibitory effect of a suppressive soil depending on the nature of the suppression[2,14].
6. Mode of inhibition. The inhibitory effect of a suppressive soil on the pathogen may be either fungistatic or fungitoxic[2,14].
7. Effect of separation by polycarbonate membrane. Whether the pathogen will be inhibited on suppressive soil when it is separated from the soil particles and microorganisms by a polycarbonate membrane (0.2 μm) can also be used as a distinct characteristic of a suppressive soil.

Similar approaches can be used to characterize a disease-suppressive soil.

These or other criteria have not been followed in other studies of the nature of inhibition in suppressive soils[1,2]. For example, Liu and Baker[15] found that soil repeatedly planted with radish became suppressive to *Rhizoctonia solani*. This was associated with increase in population of *Trichoderma* spp. which when introduced into conducive soil induced suppressiveness. However, other factors have not been ruled out and the characteristics of the *Trichoderma*-induced suppression have not been compared with that of the repeated planting. Therefore, one cannot be sure that the suppression induced by added *Trichoderma* is the induced by repeated planting.

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Biological Control of Plant Pathogens, with Special Reference to the Take-all Fungus in Suppressive Soils

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摘 要

Cook, R. J. (1985) **抑病土對小麥全蝕病菌之生物防治** （美國農部根部病害及生物防治研究室）

廣義的生物防治包括拮抗微生物，寄主植物，病原菌之變異菌系或以上任何因子之配合應用，達到抑制病原菌之接種源，感染過程或病害發展的目的。許多傳統的生物防治方法，例如輪作，耕犂，淹水及添加有機物等已不適合現代的耕作制度，故必需尋求其他的防治對策。小麥全蝕病菌（*Gaeumannomyces graminis* var. *tritici*）之生物防治即是研究抑病土作用機制發展出來的方法之一。研究結果發現，有兩類型抑病土存在，亦即針對「全蝕衰敗」（take-all decline）之專一性抑制型（specific suppression）及不論耕作歷史，任何土壤皆具有之一般性抑制型（general suppression）。對全蝕病菌而言，一般性抑制型乃是病原行腐生生存期間，拮抗微生物在病斑上或植株殘體中對病原菌的競爭作用，它影響接種源單位容積質量（植株殘體）所能引起的病斑數目，換句話說，倘若去除殘體中之拮抗微生物，則全蝕病菌之感染效率爲之顯著提高。專一性抑制型則被認爲是某類拮抗菌對病原菌之抑制作用，尤其是當這些拮抗微生物屬於侵略性的次生菌，具有抗生作用，並且和全蝕病菌，同時棲息於植株殘體時，更能顯出抑制力。能產生抗生物質之根棲螢光性細菌（*Pseudomonas* spp）是造成「全蝕衰敗」的主要拮抗菌，將它們粉衣於小麥種子上，則可阻擋全蝕病之侵襲。本文亦討論其他可能之生物防治機制，例如 dsRNA mycovirus agent 能使全蝕病菌無毒化，並且自我抑制，以及相關的 *Phialophora graminicola* 對全蝕病菌之交叉保護等。

ABSTRACT

Biological control, broadly defined, includes the use of antagonistic microorganisms, the host plant, modified strains of the pathogen, or any combination of these agents to eliminate or suppress inoculum of the pathogen, prevent infection, or limit disease development. Many traditional biological controls

(e. g. those accomplished by crop rotation, tillage to accelerate biological destruction of inoculum, and flooding, and organic amendments) no longer fit with modern farming practices. Approaches to biological control of take-all illustrate some alternatives developed for one disease where traditional controls have become impractical or obsolete. The controls under development were first recognized for their potential through studies of the mechanisms of suppression of the pathogen, *Gaeumannomyces graminis* var. *tritici* in certain soils. At *least* two kinds of suppressive soils exist; so-called "specific suppression," responsible for take-all decline; and "general suppression," operative to one degree or another in all soils regardless of cropping history. It is suggested that general suppression results from competitive effects of the succession of secondary colonists in the lesion and continuing in the infested crop residue during saprophytic survival of the fungus. General suppression affects the number of root infections per unit mass of infested crop residue (infection efficiency), which increases significantly upon elimination of competitors for the foodbase. Specific suppression acts against a background of general suppression, and is thought to also result from inhibitory effects of secondary colonists. However, the antagonists responsible for the specific suppression are thought to be more potent in their effect, being especially aggressive as secondary colonists of lesions, able to inhibit the pathogen by antibiotic action, and more likely to carryover as cohabitants with the pathogen in the crop residue. Antibiotic-producing root-colonizing fluorescent pseudomonads are implicated as the important agents in the specific suppression responsible for take-all decline. These antagonists have been shown to provide significant protection of wheat when applied as a living seed treatment. Other potential mechanisms of biological control discussed are dsRNA mycovirus-like agents in the take-all fungus, the tendency of the fungus to convert to avirulence and to inhibit itself, and cross protection by the related *Phialophora graminicola*.

INTRODUCTION

Biological control is used in this paper in the broad sense to include pathogen control by microorganisms, the host plant, or even by the pathogen used against itself[13]. A microorganism may be inhibitory to the pathogen by its ability to produce one or more antibiotics, it may be a hyperparasite (or hyperpathogen) with ability to weaken or destroy cells of the pathogen, or it may compete with the pathogen and thereby retard the growth and/or reproduction of the pathogen on or within the host. The host may play a passive role in biological control, by providing the sites for interaction between the antagonist(s) and pathogen; or it may play an active role by resisting the pathogen. Traditionally, the resistance of plants used against pathogens has been obtained by breeding, but resistance can also be induced by inoculation of the plant with certain nonpathogens; either way biological control come into play. The use of a

pathogen for its own biological control includes mainly modified or avirulent strains as carriers of infectious agents (e. g. a dsRNA mycovirus), as competitors in the infection court of the pathogen, or to induce resistance. Ultimately, we may find that modified pathogens will be the best agents of biological control.

While a concept of biological control that includes the use of antagonists, the host, or the pathogen itself may seem too broad[17] there is no logical reason to limit the concept to a more narrow definition[12]. Some would exclude the host as an agent of biological control, but as F. G. H. Lupton[35] states "accelerating or diverting evolutionary processes in order to obtain genotypes adapted to [man's] needs are a most important example of the application of biological control to agricultural and horticultural crops." On the other hand, a concept so broad can be unwieldy, and to avoid this Cook and Baker[13] proposed three subdivisions: biological control of pathogen inoculum; biological control of plant infection; and biological control of disease development after infection. These three approaches fall generally, but with some overlap, into biological control remote from the host, on the host, and inside the host.

The first approach includes those biological controls aimed at reduction of inoculum density in soil, e. g. by crop rotation, tillage to accelerate biological breakdown of crop residue infested with pathogens, or the introduction of hyperparasites to destroy pathogen spores and sclerotia in soil or in host tissue. This approach may also include the use of agents to lower the virulence of pathogen inoculum, e. g. dsRNA mycovirus-like agents to convert virulent *Endothia parasitica* in chestnut cankers to a hypovirulent state, whereupon the canker heals.[2] The second category—control of plant infection—includes any use of nonpathogenic microorganisms in the rhizosphere or on any phylloplane to preempt, exclude, or inhibit pathogens and thereby protect plants. More will be said later about this potentially very useful approach. The third proach—limit disease development after infection or attempted infection—includes the use of cross protection and induced resistance. The third approach can also include genetic improvement of the plant's resistance to pathogens, any cultural practice that helps the plant avoid or tolerate stresses and thereby maintain its resistance to weak parasites, and the use of mycorrhizae that somehow help the plant resist pathogens.

Accepting the broad concept, it is apparent that biological control is not a new way to control pathogens. Already in the 3rd century B.C. farmers in China were advised to fallow their soil if the field gave a poor yield.[13] Crop rotation allows time between the plantings of any given crop for microorganisms in soil to destroy the inoculum of pathogens that otherwise would damage that crop. Rotating crops is thus a very old as well as contemporary method of biological control. Another biological control of long standing and still used today is flooding the soil.[49] Microorganisms, probably anaerobes, are somehow involved in destruction of pathogen inoculum in flooded soil.[51] The incorporation of large quantities of organic material, e. g. barnyard manure or green manure, is an

ancient farming practice. It was shown scientifically 50-60 years ago to induce biological control of common scab of potato[42], take-all of wheat[43], and phymatotrichum root rot of cotton[30]. Organic soil amendments initiate germination followed by lysis of the pathogen propagules in soil,[40] and they increase the microbial suppressiveness of soils to pathogens.[13] Each of these familiar methods are aimed at biological destruction or suppression of pathogen inoculum in soil and therefore fit in the first category given above. Other familiar examples could be cited. It is important to realize that plant plathologists have been doing biological control research all along[12].

Unfortunately, not all pathogens can be controlled by the the traditional crop rotations, flooding, organic manural treatments, or host resistance; or these approaches no longer fit with modern farming practices or are too labor-intensive. Take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici*, fits this situation. This disease has been studied longer and more intensively than possibly any other root disease, yet it continues to be the most important root disease of wheat worldwide. It occurs in northern Europe, South Africa, Brazil, Chile, Argentina, Australia, China, Hokkaido Island of Japan, and most wheat growing areas of North America.[5] The disease can be controlled by organic amendments to the soil, but organic manures are no longer available in sufficient quantities and the practice is too labor-intensive. The disease is easily controlled by a single year of no wheat, barley, or other grass suscepts in the rotation, but the trend in cereal production, as for many other important food crops, is toward monoculture. The disease can also be controlled by tillage, but the trend in North America and elsewhere is toward less or no tillage. There is no known source of genetic resistance in wheat or barley, and the only effective chemical treatment thus far is soil fumigation, which is too expensive for wheat. Thus, for nearly the past twenty years, our USDA, ARS program at pullman has been seeking biological approaches to the control the wheat take-all fungus that will permit less or no crop rotation and hopefully less or no tillage. Our work is focused mainly on the biological mechanisms responsible for suppressive soils and take-all decline with wheat monoculture.

A mechanism of bioloical control of *G. graminis* var. *tritici* by one or some combination of each of the three approaches given above has now been identified and one method involving the introduction of antagonists of the pathgen as a seed treatment is under consideration for commercial application. The remainder of this paper will therefore be on biological control of the take-all fungus on wheat, as a means to briefly summarize the current state of this work, but also because so many principles of biological control of plant pathogens can be illustrated by the biocontrols or potential biocontrols of this fungus. The main emphasis will be on the mechanisms identified through studies of pathogen suppression in certain soils.

IMPORTANCE OF CROP RESIDUE AS A FOODBASE TO LONGEVITY AND INOCULUM POTENTIAL OF

THE PATHOGEN IN SOIL

Understanding the importance of a foodbase to the survival and inoculum potential of *G. graminis* var. *tritici* in soil is a key to understanding the mechanisms of suppression of this fungus in certain soils and how it can be controlled biologically. Like many root pathogens, *G. graminis* var *tritici* is dependent for survival between host crops on its saprophytic existence in the roots and stem bases of host plants colonized earlier through parasitism.[20] The fungus cannot effectively colonize dead substrates in competition with other microorganisms in the soil, but it overcomes this limitation by colonizing the substrates while still parts of living plants. Thereafter, the challenge to *G. graminis* var. *tritici* is one of "hanging on" to the substrates in an environment where countless saprophytes exist with ability to displace the fungus. The longer the time the fungus exists in the crop residue in soil the less the energy available for infection when a new host root finally comes along.

Wilkinson, et al[56] developed a model system to quantify the inoculum potential of *G. graminis* var. *tritici* in fragments of plant residue. Their system determined both the minimum (threshold) size of infested particles necessary for an infection to occur and also the number of infections per unit mass (infection efficiency) of different size particles. The threshold size was 250-500 μm for both infested stem bases (natural inoculum) and axenially colonized oat grains (artificial inoculum). This range compares favorably with the 420 μm estimated by Hornby[26] as the minimum size. At the threshold size and larger, the number of infections per unit weight of inoculum (infection efficiency) was the same for all particle sizes, i. e. 10 mg of particles 1-2 mm in diameter produced the same number of lesions as did 10 mg of particles either 250-500 μm or 500 μm to 1.0 m in diameter. However, the infection efficiency of artificial (infested oat-grain) inoculum was always greater than that of naturally infected stem bases.

Under field conditions, the size and total amount of infested particles at any given time after harvest will depend on the length of time since the last wheat crop was grown in the field, the frequency of wheat cropping (length of the rotation), and the frequency and kind of tillage. This is revealed by the particle size distribution of infested debris recovered in the fall after harvest and tillage of three irrigated wheat fields in Washington state. In a field cropped to wheat for the first time after rotation for several years to nonsusceptible crops, all infested particles collected 2-3 months after harvest were 12.5 mm in diameter and larger. In a second field cropped two consecutive years to wheat, most of the infested debris was again as particles 12.5 mm in diameter and larger, but in addition, an infested fraction 6.4 to 12.5 mm in diameter was recovered. In a third field, cropped three consecutive years to wheat, a large portion (most) of the infested debris again was 12.5 mm in diameter, but infested particles of many sizes down to only 0.5-1.0 mm in diameter also were recovered. The smaller size particles in the field cropped 3 years undoubtedly represented remnants of plants infected in the second and even first years that wheat was grown in the

field.

On the other hand, if the field is not tilled (seeded directly, with no prior seedbed preparation, a method gaining in popularity to prevent soil erosion), the fragments of infested crop residue are mostly as intact wheat crowns 1-2 cm in diameter[37]. Take-all in consecutive crops of wheat is therefore more severe in the Pacific Northwest of the USA if the fields are direct drilled than if intensive tillage is used. The more intensive the tillage, the smaller the particles of infested residue and the greater the portion of infested residue as particles smaller than the minimum threshold size. The greater surface area of the fragmented inoculum may also favor greater competition for the take-all fungus in the residue by microorganisms.

Wilkinson et al[58] also estimated the distance *G. graminis* var. *tritici* can grow from an inoculum particle to infect a root in different soils. The average distances for infection were estimated 1-2 mm for particles 0.25-0.5 mm in diameter, 3-4 mm for particles 0.5 to 1.0 mm in diameter and 11-12 mm for particles 1-2 mm in diameter. As expected, the estimated distances for infection increased, when the soil was pasteurized or fumigated to over 3 mm for particles 0.25 to 0.5 mm and to 5-7 mm for particles 0.5 to 1.0 mm in diameter. Particles smaller than the threshold size (90 μm to 150 μm in diameter) for natural soil were infectious in pasturized soil, but the 11-12 mm estimated as the distance for infection from particles 1-2 mm in diameter was the same whether or not the soil was treated to eliminate competing microorganisms. Wilkinson, et al[58] concluded that with the larger particles (1-2 mm in diameter), the food base was the main source of nutrients for the fungus and hence, elimination of soil organisms as competitors in the rhizosphere did not result in a significantly distance the fungus could grow to reach the root. In the smaller particles, on the other hand, nutrients in the form of root exudates were thought to be more influential which could explain why any reduction in the intensity of competition from the associated microbiota was reflected in a greater distance the fungus could grow from the smaller particles to reach a root.

CONVERSION OF SOILS FROM CONDUCIVE TO SUPPRESSIVE FOLLOWING ONE OR MORE OUTBREAKS OF TAKE-ALL

Like most root diseases, take-all increases in severity with the first two, three, or possibly four consecutive host crops. Howevre, whereas most diseases caused by soilborne pathogens eventually stabilize at a consistently severe level with monoculture of the host, or fluctuate in severity depending on conditions, take-all eventually goes into remission following one or two severe outbreaks of the disease. "Take-all decline" was first observed about fifty years ago by Fellows and Ficke[18] in Kansas in the USA and independently by Glynn[22] in England. It was not until the work of Gerlagh[21] in Holland, however, that take-all decline was demonstrated experimentally. Gerlagh[21] showed that soils conducive to the pathogen became suppressive with wheat monoculture and the presence of the take-all fungus. This pattern fits one of three categories proposed by Baker and

Cook[6] for pathogen suppressive soils, namely the pathogen establishes in soil and causes disease for a while but then severity of the disease declines. The background information and evidence for take-all decline has been reviewed by Sipton[45]. To avoid confusion with other suppressive soils the suppression responsible for take-all decline will hereafter be referred to as *specific suppression.*[21]

CHARACTERISTICS OF SPECIFIC SUPPRESSION

Specific suppression has been studied in more detail than possibly any other kind of suppressive soil. Several important features of suppressive soils and experimental approaches useful to their study are illustrated by this work and are enumerated below.

a. A bioassay for the suppressiveness is usually needed to study the suppression and, most important, to make certain that any suppressiveness demonstrated experimentally is the same suppressiveness operative in nature. Gerlagh[21] and Shipton[44] each developed bioassays, but probably the best and simplest is the pot assay of Shipton et al[46], described more fully and illustrated by Cook and Rovira [15]. In this bioassay, the field soils are diluted 1:10 or 1:100 with a standardized fumigated soil and the blend then infested with axenically colonized oat-grain inoculum of *G. graminis* var. *tritici*, potted, and sown to wheat. The plants are scored for severity of disease 4 weeks later. Soil from fields where take-all decline had occurred were the only soil suppressive to take-all in this test. D. Weller (unpublished) found it necessary to remove the first crop of seedlings and to sow again, since suppressiveness was best revealed in the second crop of seedlings. The enhanced with a second sowing and the perfect correlation between suppressiveness in the pot test with soils from fields where take-all decline had occurred is good evidence that the suppressiveness measured in the pot test is, indeed, the suppressiveness associated with take-all decline. This bioassay has since been reduced to an even easier "tube test" involving only 10 g of test soil amended with inoculum and sandwiched between layers of vermiculite in a narrow plastic tube, with one or two wheat plants per tube[56].

b. Evidence that the suppressiveness is biological usually can be obtained by the use of heat or chemical treatments to eliminate groups of organisms. In the case of the specific suppression, Gerlagh [21] with his test, and Shipton et al[46] with their pot bioassay, both showed that suppressiveness was eliminated by moist heat-treatment at 60°C/30 min. Cook and Rovira[15] showed that methylbromide and chloropicrin also eliminated the factor(s) responsible for specific suppression. The sensitivity of suppressiveness to 60°C/30 min rules out the heat-resistent, spore-forming bacteria and probably also actinomycetes[6], but leaves in contention all fungi and nonsporing bacteria as candidates to explain the effect.

c. Transferability of suppression can provide useful information on the biological nature of the phenomonon and also help reveal whether conducive soils can be made suppressive on a practical scale. The first demonstration of the transferability of specific suppression was by Shipton et al[46,6] and was done in the field. Soil from six fields in eastern

Washington was moved to a site in western Washington where it was mixed about 15 cm deep into replicate plots. The actual amount of soil incorporated was estimated at only about 0.5% by weight. Wheat seed mixed 50:50 with oat-grain inoculum of *G. graminis* var. *tritici* was then drilled into the entire plot site. Take-all was uniformly severe in all plots the first year, but was almost totally controlled by one introduced soil in the second sowing. The one effective soil was from a field near Quincy, Washington, that had been in irrigated wheat for 12 consecutive years. None of the other five soils initiated suppressiveness by the second sowing but with the third sowing, take-all decline had occurred over the entire site. This is possibly the first successful transfer of a principle responsible for a suppressive soil. The factor(s) transfered with the soil from Quincy obviously multiplied in response to the host and pathogen.

d. The site of action of suppressiveness, if known, can reveal useful information on the mechanism of action. Wilkinson et al[57] found that neither the minimum (threshold) size of infested particles nor the number of infections per unit mass of particles was affected by the microbial factor(s) responsible for specific suppression. This finding held for both natural (stembases) and artifical (axenically colonized oat grains) sources of inoculum. They showed further[58] that the estimated distance the fungus could grow from particles of infested oat grains to infect the root was the same for suppressive (specific) and conducive soils, and that the values increased about the same amount when the respective soils were pasteurized. Estimated distances were affected by microorganisms but apparently not by *the microorganisms* responsible for take-all decline. These data support an earlier demonstration in the field[11] that specific suppression operates to suppress disease progress after infection, but does not affect prepenetration activities of the pathogen. The only opportunity for the specific suppression to affect the pathogen during its saprophytic existence or prepenetration activities is when the responsible microorganisms carry over in the inoculum particles with *G. graminis* var. *tritici*[55]. This point is discussed in more detail below.

e. Persistence of the pathogen in a virulent form on the host in suppressive soil is evidence that the factor (s) in suppressive soil must be external to and act on the pathogen rather than the result of some genetic or physiological change within the fungus itself. Asher[4], in a survey of wheat fields in England, and Cook and Naiki[14], in a survey of wheat fields in the Pacific Northwest, USA, found no evidence that virulence in the pathogen population was significantly different before than after take-all decilne. In the work of Cook and Naiki[14], the pathogen was recovered from 50-100% of random plants in fields after take-all decline and 90-95% of the isolates were virulent, but still disease was mild or nonexistent.

Our evidence continues to indicate that take-all decline does not occur until take-all has occurred. Thereafter, the pathogen persists in a virulent form but is suppressed. At the western Washington plot site, take-all decline occurred in all plots by the third year, which was after

two outbreaks of severe disease. The advantage of the introduced from Quincy soil was to initiate decline already in the second crop. All of this points to a fairly specific microorganism or group of microorganisms responsible for a natural biological control of the wheat take-all fungus.

ALTERNATIVE EXPLAINATIONS FOR TAKE-ALL DECLINE

There are many forms of antagonism of the wheat take-all fungus in soil, the rhizosphere, and within roots, including destruction of hyphae by amoebae[9,25] cross protection by the avirulent or weakly virulent *G. graminis* var. *graminis* and *phialophora graminicola*[16,60] and the inhibitory effects of root-colonizing fluorescent pseudomonads[15,47,48,59]. In addition, *G. graminis* var. *tritici* is commonly infected with dsRNA mycovirus-like agents[19] and workers in France have proposed that transmission and spread of mycoviruses within the population of the pathogen causes a progressive loss in virulence within the population and hence, take-all decline.[33] Probably each of these microbiological phenomonon, and others not yet identified, affect the take-all fungus to one degree or another, but the question remaining is: which, if any, cause take-all decline[27]?

The factor(s) responsible for take-all decline must fit the following characteristics based on information presented in the previous section.

a. Multiply when introduced as a small quantity of soil into a large volume of soil (e. g. 1:100 or 1:200 dilution in soil) and wheat then grown with the take-all fungus present.
b. Operate on the root or in the lesion to suppress disease progress after infection.
c. Act with sufficient speed to suppress symptom expression on seedlings already during the first 4 weeks after planting if in a greenhouse pot [15], or retard disease spread into the crown and from seminal to coronal roots if in the field[11].
d. Be sensitive to moist heat-treatment at 60°C/30 min.
e. Permit the pathogen to remain virulent and to complete its life cycle on roots of wheat without causing serious damage to the plant.

Amoebae are too slow and inefficient to intercept the pathogen on roots and in lesions and to significantly retard disease development already within the first 2-3 weeks after sowning in soil heavily infested with the pathogen. The giant vampyrellid amoebae are active only if the soil is drained (drier than −0.01 to −0.05 matric potential) but not drier than −0.2 to −0.25 bars.[24] Even soil at "field capacity" (about −0.3 bars) is too dry for amoeboid activity. Finally, amoebae are most active in soil at pH 6.5 to 7.5[24], yet this pH range is where take-all is most severe. If amoebae are responsible for take-all suppression, then expectedly there should be less disease where amoeboid activity is greatest. The major effect of amoebae is probably as a predator on hyphae during saprophytic survival of the fungus[9], rather than an interceptor of the fungus during its parasitic activity.

Cross protection by *P. graminicola* cannot explain take-all decline on the basis that populations of this antagonist tend to decline rather than increase

during wheat monoculture.[16] This fungus is widely distributed in England in grass swards, where it exists as an inhabitant and weak parasite of grass roots under cool conditions. *P. graminicola* colonizes the roots of the first wheat crop sown after a grass sward, but with successive crops of wheat, its population declines while that of *G. graminis* var. *tritici* increases. The take-all suppression by *P. graminicola* is therefore less effective with continuing monoculture, a pattern very different from take-all decline.

Hypovirulence caused by dsRNA mycovirus infections cannot explain take-all decline on the basis of two studies, both which showed that the pathogen population is still 90-100% as virulent isolates after take-all decline[4,14]. This does not mean that hypovirulence and dsRNA mycovirus infections cannot co-ntrol take-all if properly understood and exploited. Besides hypovirulence, the fungus may also convert from virulent to avirulent[38], possibly because of a self inhibitor[39] described by Romanos et al [41]. These genetic and physiological mechanisms deserve more study as a means to biocontrol of the take-all fungus, but they are seemingly unrelated to the microbiological factor(s) that act on the fungus to bring about take-all decline.

The only form of antagonism that can explain take-all decline is the inhibition of the wheat take-all fungus on roots and in lesions by the fluorescent pseudomonads. These ubiquitious bacteria are sensitive to 60°C/30 min and are common colonists of plant roots and soil surrounding plant roots. They also produce many antibiotics as well as siderophores[31] and have been shown to colonize roots and inhibit plant pathogens [32,50]. In addition to this circumstantial evidence, there is now direct evidence for their role in take-all decline.

The first evidence that certain strains of *Pseudomonas fluorescens* were involved in take-all decline was obtained by Cook and Rovira[15]. They used the pot assay of Shipton et al[46] but substituted soil cultures of individual strains of bacteria for the field soil added to the fumigated soil. Two hundred strains of bacteria were tested; 100 came from a collection maintained by A. D. Rovira and 100 came from roots of wheat from earlier pot assays with conducive and suppressive soils. Only eight of the 200 soil cultures diluted 1:100 with fumigated soil gave suppression of *G. graminis* var. *tritici* that matched the characteristics of specific suppression as revealed in the pot assay, and of the eight, all were from the group isolated originally from roots of wheat growing in a 1:100 dilution of suppressive soil. Of these eight, seven were fluorescent *Pseudomonas* sp.

Weller[13] isolated bacteria from roots of a second sowing of 4-week-old wheat seedlings grown in conducive and suppressive soils diluted 1:10 with fumigated soil and amended with oat-grain inoculum of *G. graminis* var. *tritici*. Different strains of bacteria seemed to dominate different roots, but the predominant and commonly only bacterial type at the 10^{-4} and 10^{-5} dilutions of macerated roots was fluorescent *Pseudomonas* sp. The total number of fluorescent *Pseudomonas* sp. were about 1 log greater (e. g. 10^5 vs 10^4) on roots of seedlings growing in suppressive compared to those on roots of wheat growing in conducive soils.

Equally significant, 30-55% of those from roots growing in suppressive soil were inhibitory compared with only 10-20% of those from roots growing in conducive soils[13]. Since the total number of fluorescent pseudmonads was about 10 times greater and the number inhibitory was at least two times greater, Weller's data indicated that the total number of inhibitory bacteria could be as much as 20 times greater on the roots in suppressive than on roots in conducive soil.

In another study, random isolates of *P. fluorescens* from wheat roots were tested both in vitro, for antibiotic activity against the wheat take-all fungus on agar media, and in vivo, for ability to suppress take-all in the tube test when applied as a seed treatment[54]. Isolates most inhibitory in vitro were the most suppressive to take-all in vivo, and those least inhibitory or noninhibitory in vivo were the least suppressive or nonsuppressive in vivo. Again, a significantly higher ($P=0.05$) frequency of inhibitory (and take-all suppressive) isolates of *P. fluorescens* were obtained from roots growning in the suppressive soil compared to conducive soil[54]. All of these data indicate a qualitative as well as quantitative shift in the population of fluorescent pseudomonads toward both a higher total number and especially a higher number antibiotic to *G. graminis* var. *tritici*.

Wong and Baker[59] concluded from studies with isolates of pseudomonads added to soil at about 10^7 cfu/g that inhibition by antiboitic production in vitro was not a reliable indicator of ability to suppress take-all in soil. We have also found isolates of fluorescent pseudomonads that suppress take-all in vivo but not in vitro, and vice versa. However, it cannot be concluded on the basis of such results that antibiotic production is not an important trait to strains inhibitory both in vitro and in vivo; our results continue to indicate that of strains with antibiotic-producing ability and also effective in vivo are dependent for their effectiveness in vivo on this antibiotic-producing ability.

A MODEL TO EXPLAIN TAKE-ALL DECLINE

Wilkinson et al[55] performed a highly significant experiment that makes it possible to propose more precisely how take-all decline comes about. Inhibitory-positive (wild-type) and -negative (mutant) strains of *P. fluorescens* were applied as a cell suspension in each of three ways: to natural soil at abouut 10^6 cfu/g soil, as a seed treatment at about 10^7 cfu/seed, and infiltrated into particles of the oat grain inoculum used to infest the soil. The bacteria added as cells to the soil were virtually without effect on take-all. In contrast, significant suppression occurred with the bacteria as a seed treatment and greatest suppression occurred with the antagonist infiltrated into the inoculum particles. In all cases, however, no disease suppression occurred with the inhibitory-negative isolates, even if infiltrated into the inoculum particles. These results confirm the importance of antibiotic-producing ability of the bacteria, but suggest further that the greatest inhibitory effect of these bacteria occurs when they exist as cohabitants with the pathogen in the inoculum fragments.

Following a break of one or more years with no wheat or barley, most

inoculum of *G. graminis* var. *tritici* will have displaced in the crop residue fragments by saprophytic microorganisms. However, a few fragments with viable hyphae of the pathogen will still be present and available to infect roots of the next crop. Since isolates that are highly virulent also survive the longest time[10], the inoculum available after a break is likely to be the most virulent of the original population carried over from the last wheat crop[38]. The most successful infections are also likely to originate from inoculum in crop residue lacking inhibitory isolates of *P. fluorescens*. Since inhibitory fluorescent pseudomonads scattered in soil are also unlikely to protect roots against the fungus, even if at 10^6 cfu/g[55], infections of the first crop after a break, while rare, are likely to be severe. These infected plants will then serve as a larger inoculum source for the next crop.

With the second sowing, more plants will be infected, and infections are again likely to be severe. However, fluorescent pseudomonads are also likely to be establishing in higher populations with the second and subsequent sowings, as secondary colonists of the "leaky" lesions caused by the take-all fungus. Of all secondary colonists, fluorescent pseudomonads seem especially attracted to the lesions. A shift toward a higher frequency of antibiotic types might be explained on the basis that inter and intra species competition among microorganisms in the nutrient-rich lesion may be so intense that gradually the most inhibitory types win out. These types then become cohabitants with the take-all fungus in the crop residue where they are especially effective and also then poised to re-establish and multiply in the next lesion caused when hyphae of *G. graminis* var. *tritici* emerge from the reside in response to roots of the next crop. This cycle is then repeated until an equilibrium is reached between inhibition of *G. graminis* var. *tritici* on the one hand, but enough-infected tissue to support the inhibitory bacteria, on the other hand.

This model can account for the quanitative as well as qualitative shift in population of root-colonizing fluorescent pseudomonads and also why virulent *G. graminis* var *tritici* can be recovered from roots of up to 100% of wheat plants in fields after take-all decline. It can also account for the observation that severe take-all usually must occur before take-all decline will occur; the more severe the infection. the more abundant the sites and nutrients for multiplication of bacteria and the greater the selection pressure in favor of inhibitory bacteria. However, the best direct evidence to support this model is the finding of Weller[13,54] and also W. Howie, (unpublished), that marked strains of *Pseudomonas fluorescens* inhibitory to *G. graminis* var. *tritici*, when monitored as root colonists following their introduction on seeds, achieve the highest populations and persist at the higher populations longer if *G. graminis* var. *tritici* is also on the roots. The bacteria are highly favored by the presence of the wheat take-all fungus on wheat roots, possibly because they respond to nutrients liberated from lesions. Work is now progressing to establish more conclusively the validity of this model.

APPLICATION OF ROOT-COLONIZING

FLUORESCENT PSEUDOMONADS FOR TAKE-ALL SUPPRESSION

The transfer of whole untreated soil from field to field is obviously impractical on a commercial basis. Even a seemingly small amount of soil, e. g. 0.5% by weight, would amount to over 800 mt of soil/ha. The logistical problems of mass rearing antagonistic fluorescent pseudomonads in soil culture and applying this would be even more insurmountable, even if the soil inoculum was applied in the seed row only. The weight of soil would be several times the weight of any dry fertilizer now applied with and beneath the seed by commercial drills. The most practical approach would be a seed treatment.

When the strains of fluorescent pseudomonads inhibitory to *G. graminis* var. *tritici* in vitro were applied to wheat seed and the seed planted in plots together with oat-grain inoculum of the pathogen, the degree of take-all suppression was highly significant[53]. The introduced strains colonize the roots and compete well with native rhizosphere colonists[52]. In some trials, a mixture of strains was more suppressive than a single strain. The treatment is now under test in the field for the seventh consecutive year, including in commercial fields seeded by growers. Field tests are conducted only where take-all is the main yield-limiting factor. Yield increases of 10-25% (significant at $P=0.05$, sometimes at $P=0.01$) have been obtained in approximately two trials out of every three. Unfortunately, the treatment has failed about 1/3 of the time which indicates that either soil conditions are not always satisfactory for root colonization by the introduced strain(s) or that improved strains are needed. Nevertheless, the trials are encouraging and the treatment is now under consideration for commercial application.

The potential for seed bacterization as an approach to biological control of root pathogens is also indicated by recent accomplishments of other investigators with other antagonistic strains of bacteria. A significant breakthrough was by Broadbent et al[7], who's *Bacillus subtilus* strain A13, antagonistic in vitro to all nine test fungi, was shown to increase tillering, accelerate maturity, and increase yields of wheat, barley, and oats when applied as a seed treatment[36]. This same strain as a seed treatment also increased yields of sweet corn and carrots[36]. More recently, Burr et al[6] and Kloepper et al[31] showed that strains of fluorescent pseudomonads isolated originally from roots or tubers of potatoes and inhibitory in vitro to a wide spectrum of bacterial and fungal pathogens increased growth and yield of potatoes in the field in California when applied on the potato seed pieces. The same or similar strains also increased yield and sugar content of sugar beets when applied as a beet-seed treatment[50]. Biological seed treatments have the potential to become a practical method by which plants are protected from root pathogens. All evidence indicates that the bacteria grow or are carried downward with the roots and that they protect roots against pathogens by their competitive and inhibitory ability.

A unique feature of our method is that we isolate the antagonists from roots of wheat growing in soil suppressive

to *G. graminis* var. *tritici*. Effective antagonists can be isolated from roots growing in conducive soil, but the odds of obtaining effective strains is much better (possibly several times better) if the soil is from a field that has undergone take-all decline. In addition, we target our selection process for strains with ability to inhibit the take-all pathogen. Any increased growth response of wheat to the presence of one or a mixture of our strains is the result of suppression of the take-all fungus and not a stimulation of the plant[53].

SUPPRESSIVENESS OF SOILS TO G. GRAMINIS VAR TRITICI NOT RESPONSIBLE FOR TAKE-ALL DECLINE

Gerlagh[21] was the first to distinguish between the suppressiveness of soils that developed in response to severe take-all and the suppressiveness to this fungus characteristic of all soils regardless of their cropping history. He referred to the latter type as *general suppression*. All soils are suppressive to one degree or another to all soilborne pathogens[13] and *G. graminis* var. *tritici* is no exception. The fact that soils become progressively more suppressive with increasing soil temperatures up to 25-30°C[23] may be an example of an enhancement by the higher temperatures of Gerlagh's general suppression[15].

Alabouvette et al[1] and Hwang et al[28] demonstrated that the suppressiveness of certain soils to Fusarium wilt pathogens relates, in part, to greater competition for nutrients between the *Fusarium* and soil microorganisms. More nutrients as glucose was needed for chlamydospore germination in the wilt-suppressive than in the wilt-conducive soil. The possibility that general suppression of soils to the wheat take-all fungus is related to more intense competition for nutrients during prepenetration growth[13] was therefore tested (Alabouvette and Cook, unpublished). The amound of microbial biomass in the soil and available to respond to an introduced source of carbon and energy (such as contained in root exudates) was estimated by both the Jenkinson[29] and Anderson and Domsch[3] methods. Each soil was infested, respectively, with five different sizes of oat-grain particles between 0.25 μm (threshhold) and 1.0 mm in diameter and at concentrations of 1-10 mg/g soil. The number of lesions per plant was counted on 4-week-old seedlings and the number of lesions per unit mass of inoculum (infection efficiency) was then calculated. It was expected that the greater the total microbial biomass available to compete with *G. graminis* var. *tritici* for nutrients (exudates in the rhizosphere), the fewer the number of infections per unit mass of inoculum.

As found by Wilkinson et al[57], the infection efficiency of any given particle size varied greatly among soils but was not be related to whether or not the soil came from fields where take-all had declined. On the other hand, without exception, the infection efficiency was significantly greater in pasteurized than in the same soil used without treatment, suggesting that microorganisms suppressive to *G. graminis* var. *tritici* occurred in all soils. However, there was also little or no relationship between infection efficiency of inoculum in any given soil and the total microbial biomass of the soil as measured by either of the Jenkinson[29] or Anderson and Domsch[3]

methods. For example, the values for infection efficiency were lowest in a noncropped (virgin) Ritzville silt loam having only about 1% organic matter content and registering a very low total microbial biomass, and were highest in an organic garden soil with a very high total microbial biomass, Other soils registering a relatively low microbial biomass supported a relatively high infection efficiency, and vice versa. The addition of increasing rates of inorganic N up to 100μg N/g soil had no significant effect on the values in any soil. Obviously, some mechanisms other than competition for exogenous nutrients from total microbial biomass active during prepenetration growth of *G. graminis* var. *tritici* must be operative.

A MODEL TO EXPLAIN GENERAL SUPPRESSION

Soilborne plant pathogens depend for their carbon and energy supply during prepenetration in the rhizosphere on both their endogenous reserves (within the propagules) and exogenous nutrients (supplied as root exudates). If their main carbon and energy supply is exogenous, then competition from the total microbial biomass active in the rhizosphere should be most important. On the other hand, if the main carbon and energy supply is endogenous, then microbial activity in and around the propagule itself should be most important source of competition. Wilkinson et al[58] concluded that for *G. graminis* var. *tritici*, the larger the inoculum particle the less dependent the fungus on exogenous nutrient supplies and the more it operates on nutrients available in its foodbase. This raises the possibility that general suppression of *G. graminis* var. *tritici* is the result of colonists of the foodbase of *G. graminis* var. *tritici* rather than competitors for the root exudates. Lin and Cook[34] have already demonstrated this as the mechanism of suppression of *Fusarium avenaceum*. *Trichoderma*, *Mucor*, and *Penicillium* species were shown to colonize the foodbase of this pathogen and thereby keep it from infecting lentils. These and other fungal colonists of dead plant remains are cosmopolitan in soils, which can account for the general suppression of all soils to *G. graminis* var. *tritici*. Moreover, these colonists would be eliminated by 60°C moist heat treatment of soil, which can account for why the infection efficiency of inoculum particles is greater in all soils when pasteurized compared to when tested nontreated. The different degrees of general suppression in the different soils may be the result of differing amounts of activity of these fungi in the soils, determined by many biotic and abiotic factors.

Chlamydospores of *F. oxysporum* arc only about 10 μm in diameter and probably supply only a small portion of the total nutrient used by the fungus for germination and growth prior to infection of the root. This may explain why the suppressivenss of soils to pathotypes of this fungus can be overcome by adding more nutrients to the soil[1,28]. The soil microbiota acts as a sink for carbon and energy released as root exudates and the larger and more responsive microbiota, the greater the sink and hence the greater the suppressiveness of the soil to *F. oxysporum*. In contrast, a pathogen such as *Sclerotium rolfsii*, having propagules 1-2 mm in diameter, presumably

would be more vulnerable to competition for its endogenous reserves by colonists of its sclerotia than to the magnitude of the sink for nutrients released a exudates. Substances released from roots may serve to initiate sclerotial germination and may help determine the direction of hyphal growth, e. g. toward the root, but would not be a significant component of the total nutrient supply used by the fungus to reach and infect the root. Probably the inoculum particles of *G. graminis* var. *tritici* are more like the sclerotia of *S. rolfsii* than the chlamydospores of *F. oxysporum*. The nutrient supply in the fragments of crop residue occupied by the fungus is in essence, an endogenous supply for this fungus. It is therefore not surprising that Alabouvette and Cook (unpublished) found no relationship between total microbial biomass of the soil and suppressiveness of the soil to this fungus.

SECONDARY COLONISTS: THE COMMON FACTOR IN THE EXPLAINATIONS OFFERED FOR SPECIFIC AND GENERAL SUPPRESSION

G. graminis var. *tritici* is vulnerable during both its parasitic and saprophytic existence to the competitive and inhibitory effects of secondary colonists. Many bacteria and fungi can be readily demonstrated to occur in lesions caused by this pathogen on wheat and barley. Indeed, they interfere significantly with isolation of the pathogen from infected tissues onto agar media. The older the lesion, the more difficult it is to recover the pathogen, even from infected tissues that are surface sterilized. Secondary colonists continue to pose a problem as competitors of the take-all fungus during its saprophytic survival.

Specific and general suppression both are thought to result from inhibition of *G. graminis* var. *tritici* secondary colonists of the substrate occupied by the pathogen. The main difference between the two types is as follows: General suppression is thought to result from competition from many microorganisms for nutrients in the foodbase, including from saprophytic colonists of the crop residue occupied by the pathogen, but probably also from competitors established already as secondary colonists of the lesion. General suppression could even result from a succession of colonists of the inoculum particles beginning during the parasitic phase and continuing during the saprophytic existence of the fungus. Specific suppression functions against a background of general suppression, i. e. in addition to the competitive effects of a succession of colonists of the substrate of *G. graminis* var. *tritici*, antibiotic-producing fluorescent pseudomonads in particular are thought to become increasingly more dominant, in the lesion first but then carried over as cohabitants with the pathogen in the foodbase. Rather than suppression by competition, specific suppression is thought to result from suppression by antibiosis, and to result from bacterial antagonists rather than fungi.

Obviously, much more work remains to be done to validate or dismiss these explanations for the two kinds of suppression of the wheat take-all fungus in natural soils. Whether we know the mechanisms or not, work can proceed on the use of pseudomonads as a seed treatment to protect wheat against take-

all, and also to find ways besides tillage to encourage secondary colonists of the foodbase used by this fungus for survival and infection. Work should also proceed on the genetic mechanisms of avirulence, hypovirulence, and self inhibition, on the chance that these also can be exploited for biocontrol purposes. Cross protection by *P. graminicola* is still another possibility; it is already working in certain rotations in England and might work elsewhere if the proper strains and methods for their introduction could be found[60]. Successful biological control of the wheat take-all fungus will ultimately depend on several approaches and not just a single approach.

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Problems and Prospects of Rhizoctonia Diseases and Their Control in Japan

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摘　　要

Ui, T. (1985) 日本 **Rhizoctonia** 病害之問題與前瞻及其防治　（日本北海道大學）

日本產 *Rhizoctonia solani* 可分爲 8 個菌絲融合羣 (anastomosis group, AGs)，部分融合羣並具有亞羣 (subgroups) 。各 AGs 之菌株是以DNA-DNA 再配對動力學 (DNA-DNA reassociation kinetics) 之方法研究其遺傳純一性 (genetical homogenicity) 。具植物病原性的 Rhizoctonias 屬於 AG 1-5，這些菌株均由北海道的耕地及未耕地的植物及土壤中分離而得。由 *R. solani* 引起的土壤傳播性病害遍佈日本，如各類植物的猝倒病、馬鈴薯的黑痣病、甜菜的根病等。雖然有 10種以上之藥劑被用以防治 Rhizoctonia 病害，但除非使用燻蒸劑，否則難以完全根除土壤中的接種源。

本報告討論 *R. solani* 的生物學及以紙盆種植甜菜直接移植方式 (paper pot transplanting system) 防治 Rhizoctonia 病害之可行性。AG 4 及 AG 1-B 爲引起幼苗猝倒病的病原菌，可以 PCNB 混於紙盆的上層土中而加以防治。AG2-2 引起的根腐病在盛夏發生，以殺菌劑施於根頸 (crown) 可達到防治效果，而以 PCNB 及滅普寧 (mepronil) 最爲有效。AG 2-2 的擔孢子可引起葉枯病，噴洒滅普寧及依普同 (iprodione) 殺菌劑可有效予以防治。紙盆移植方式廣爲採用後，甜菜 Rhizoctonia 病害尤其是根腐病顯著的減少。此法不但可更經濟有效的使用化學藥劑，而且可大大減少土壤中接種源的密度。

由 AG 2-2 引起的根腐病在甜菜連作田中初期發病嚴重，隨後病勢減輕。此種能使病害減少的土壤對 AG 2-2 有顯著的抑制效果。因此紙盆中加入此種土壤可能是有效的生物防治方法。

ABSTRACT

Rhizoctonia solani in Japan is divided into 8 anstomosis groups (AGs), and some of them include subgroups. The genetical homogenicity in the isolates of respective AGs has been studied by DNA-DNA reassociation kinetics. Plant

pathogenic Rhizoctonias belong to AG 1-5, and they are isolated from the plants and soils in cultivated and uncultivated areas in Hokkaido. The widely distributed soil-borne diseases caused by *R. solani* in Japan are damping-off various plants, black scurf of potatoes, root rot of sugar beets, etc. More than ten fungicides have been registered for the control of Rhizoctonia diseases, however, complete eradication of soil inoculum is difficult except by fumigants.

In this report biology of *R. solani* and control of Rhizoctonia diseases of sugar beet grown under paper pot transplanting system were discussed. AG 4 and AG 1-B are the pathogenic AGs to cause seedling damping-off. They are controlled by PCNB mixed with upper layer of paper pot soil. Root rot occurring in midsummer is caused by AG 2-2 and it is controlled by the spray of fungicides to crown. PCNB and mepronil are generally effective. Basidiospores of AG 2-2 cause the foliage blight. Spray of fungicides, such as mepronil and iprodione is most effective. Rhizoctonia diseases of sugar beet, especially root rot decreased remarkably, after the paper pot transplanting system was widely adopted. The system makes it possible to apply chemical control economically and effectively, and the inoculum level of *R. solani* in soil can be greatly reduced.

The root rot caused by AG 2-2 declines, after an initial severe outbreak, during monoculture of sugar beet. The disease declined soil is remarkably suppressive to AG 2-2, and it might be possible to use such soil in paper pots for effective biological control of the disease.

A brief history of soil-borne diseases and their control in Japan

Since the 17th century, Japan's agriculture has progressed rapidly centering around the cultivation of rice. Barley, wheat, rape and some vegetables were cultivated as second crops to rice in paddy field after drainage of the soil. It is probable that there were few serious problems of root diseases of upland crops grown under such conditions, because rapid buildup of soil-borne pathogens in the soil must have been prevented by repeated flooding and drainage. In the 18th century, the intensive cultivation of tobacco, cotton, and vegetables in the central parts of Japan was attended by the increase of soil-borne diseases. In the agricultural classics published in those days, there were several descriptions on the symptoms of soil-borne diseases attributed to soil-sickness or inadequate crop sequences, and long term rotation with different crops was recommended.

Japan's plant pathology dates from the last decade of the 19 th century, and diseases of rice had been the most important research subjects until 1953 when organic mercury fungicides achieved a great success in the control of rice blast. During those days, research on soil-borne diseases was carried out sporadically. There were two epoch-making control techniques: control of Fusarium wilt of watermelon by resistant

rootstocks reported by Murata and Ohara in 1936[12], and control of Cephalosporium stripe of wheat by flooding of wheat field reported by Ikata and Yoshida in 1937[5].

Another interesting method was the application of Trichoderma spores into soil, From studies on the antagonistic action of microorganisms carried out by Nishikado and associates during the 1940s, "Trichoderma Dust" was developed. The dust developed by Ohshima, one of Nishikado's associates contained spores of *Trichderma lignorum* (10^8/g) and was registered in 1956 for the control of stem rot of tobacco caused by *Sclerotium rolfsii*.

Anastomosis groups of *Rhizoctonia solani* in Japan

In the extensive studies on the physiological specialization of *R. solani*, Matsumoto[11] concluded in 1923 that "the fusion of hyphae may take place only between the hyphae from strains which are closely related" and later he pointed out that the hyphal anastomosis can be used as a criterion for the recognition of *R. solani* and *Corticium sasakii*. Hyphal anastomosis is now widely accepted in the grouping of *R. solani* and eight such anastomosis groups (AGs) have been recognized in Japan (Table 1). From the

Table 1. Anastomosis groups of *Rhizoctonia solani* found in Japan

Anastomsis[1] Group	Subgroup	Ecological group[3]
AG-1	I-A[3]	sheath blight type
	I-B	web blight type
AG-2-1		winter crop type
AG-2-2	IIIB[3]	rush type
	IV	root rot type
AG-3		potato type
AG-4	HG-I[5]	praticola type
	HG-II	
AG-5		
AG-6	HG-1[5]	
	GV	
AG-7[2]		
AG-BI[4]		

1) Ogoshi (1976) 2) Homma et al (1983)
3) Watanabe & Matsuda (1966) 4) Kuninaga et al (1978)
5) Kuninaga & Yokosawa (1984)

evidence on DNA base composition of *R. solani*, each of the AGs is considered to be genetically independent unit[9].

Some AGs were each divided into subgroups according to cultural and pathological characteristics: AG 1 includes 3 subgroups; AG 2-2, two subgroups; AG 4, two subgroups, etc. The genetical relationship between AGs and their subgroups was ascertained by DNA base

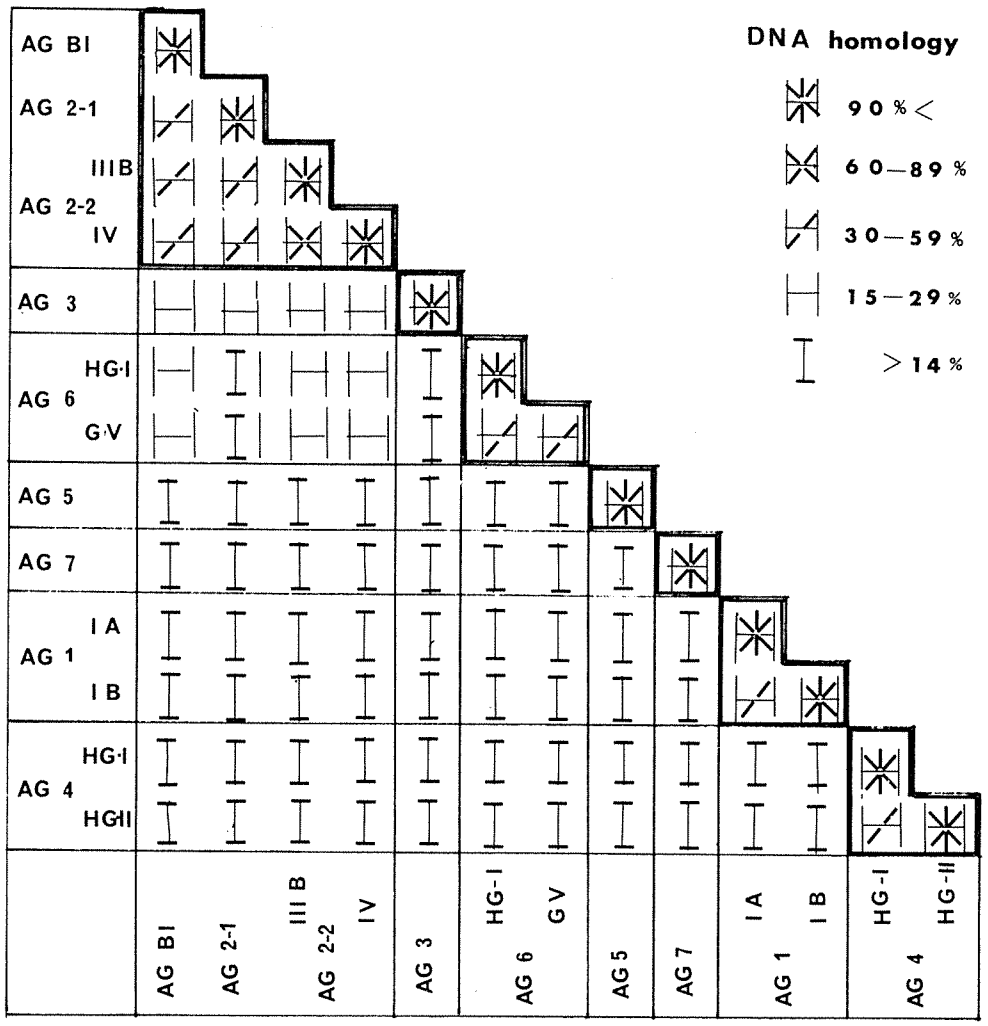

Fig. 1. DNA base sequence homology in the anastomosis groups (AGs) of *R. solani* (Courtesy of S. Kuninaga)

sequence homology[10] (Fig. 1).

AGs in Hokkaido: Anastomosis test of *R. solani* obtained from reserved forests and uncultivated areas in Hokkaido revealed that AG 1, AG 2, AG 3, AG 5, AG 6, and AG BI were native inhabitants of Hokkaido. There are several AGs in cultivated soil and the dominant AG varies with the crop cultivated: i.e., the dominant one in bean field is AG 5 and in sugar beet field AG 2-2. When sugar beet is cultivated for years under monoculture, the diversity distribution pattern of AGs in soil is gradually simplifyed and eventually almost all the isolates in soil belong to AG-2-2. During the monoculture of sugar beet usually the root rot declines gradually.

Rhizoctonia diseases of sugar beet and pathogenic AGs

Sugar beet can be infected with *R. solani* at the different stages of its growth, and various symptoms are

observed: damping-off in seedling stage, root rot or dry rot canker in big tap root observed in the mid-summer, and occasional root rot of stecklings in the propagation farms. Foliage blight appears in the summer when the temperature and humidity are high.

Several AGs were isolated from diseased seedlings grown in paper pots, and they were AG 1-B, AG 2-2, AG 4, AG 5. Binucleate *Rhizoctonia* (*Ceratobasidium* spp.) was most frequently isolated

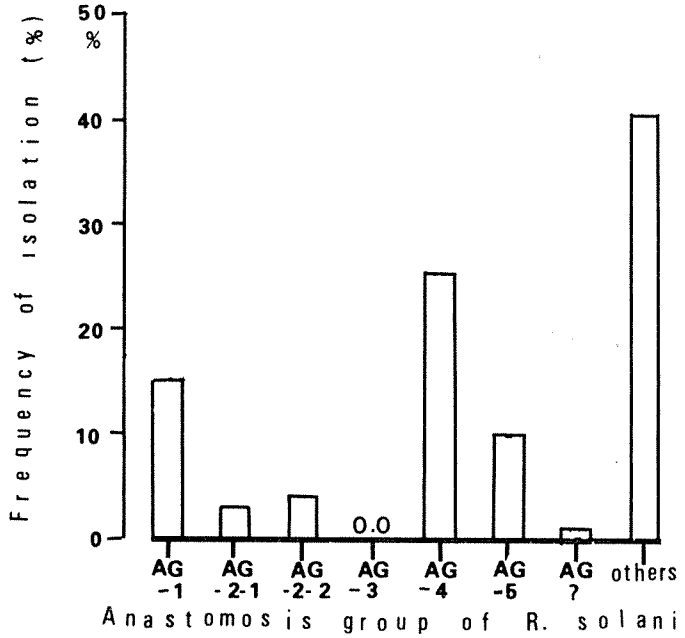

Fig. 2. Frequency of AGs isolated from sugar beet seedlings grown in paper pot. Others=binucleate Rhizoctonia

(Fig. 2). Among them, AG 1-B, AG 2-2, and AG 4 are most virulent, while AG 2-2 was isolated infrequently and caused occasional damping-off in the seedlings grown in paper pots. Paper pots infested with AG 2-2 should not be transplanted, because they constitute inoculum sources for root diseases in the summer, From these results it is concluded that the pathogenic AGs causing damping-off are mainly AG 4 and AG 1-B.

From the crown and tap roots of sugar beet in various fields, AG 2-2 was most frequently isolated, and AG 1-B which caused root rot of steckling was rarely encountered. The inoculation experiments with different AGs in sugar beet fields, the typical sympotms of root rot were induced by AG 2-2. The most suitable method of inoculation was the application of dried grain inoculum of AG 2-2 to soil surface within 3 to 5 cm from the crown, between mid June to early July[14]. Infection of tap roots was caused by the hyphae growing out from inoculum infecting petioles or crown.

After the roots were infected, sclerotia were produced on their surface and in soil around diseased roots. By the sieving-floatation method, 14.3-25.0 and 8.3-10.0 sclerotia/g dry soil were detected

from rhizosphere and non-rhizosphere soils. Generally, more than 80% of sclerotia were in the upper 10 cm of soil and within 10 cm from the diseased root[15].

The germination rate of sclerotia extracted from soil of diseased field was 83% at harvest time, and was 27.6% in the following spring[2]. AG 2-2 was also isolated from particulate plant debris in soil from same field. The average of isolation rate was as high as 35% at harvest time, and extremely low at 1.7% in the following spring. These results pointed out that sclerotia of AG 2-2 which survive in soil are the main inoculum of the following year. Pathogenicity of AG 4, occasionally isolated from soil, was limited to the juvenile tissues of sugar beet.

Basidiospores of AG 2-2 are produced on the surface of petioles and soil, when disseminated, they infect leaf blades, causing small primary lesions. From the lesions hyphae are growing out and invade the epidermis of leaves leading to the appearance of typical symptoms of foliage blight in August when the temperature and air humidity are high. Occasionally AG 1-B was isolated from the primary lesions, only in the early stage of disease.

Therefore, in controlling Rhizoctonia disease of sugar beet, the target AGs are AG 4 in the case of damping-off of seedlings and AG 2-2 in both root rot and foliage blight.

Fungicides for the control of Rhizoctonia diseases

The earliest records of Rhizoctonia diseases are demping-off of eggplants in 1903 and black scurf of potato in 1902 from Hokkaido. Since then, there were many experiments on the control of the diseases, however few of recommended methods were practised. PCNB was the first fungicide introduced to Japan being effective against Rhizoctonia diseases, and it was registered in 1961.

Several anti-Rhizoctonia fungicides were developed in the 1960s, after the outbreak of epidemics of rice sheath blight caused by *R. solani* AG 1. They were organic arsenic compounds and antibiotics. Among them, validamycin A was registered for soil-borne Rhizoctonia diseases, and it is recommended for bottom rot of lettuce, sore-shin of tobacco, and for seed potato treatment. However, it was not effective enough to control black scurf of potato in some fields in Hokkaido where abundant inocula remaind in soil.

There are many fungicides with broad fungicidal spectra, registered for the control of seedling damping-off. Generally, the seed furrow application of these fungicides is effective when the plants are young. Recently, fungicides which are effective against diseases caused by pathogenic Basidiomycetes have been developed. Among them, mepronil was registered for control of damping-off of vegetables, foliage blight and root rot of sugar beet, etc. Iprodione, a fungicide especially effective against Sclerotinia and Botrytis diseases, was registered for controlling Rhizoctonia foliage blight of sugar beet.

Chemical control of Rhizoctonia diseases of sugar beet

Since the paper pot transplanting method in sugar beet cultivation was

Table 2. Activity of fungicides damping-off of sugar beet caused by four AGs of *R. solani* (source: Ass. Hokkaido Sugar Beet Tech., 1978)

Fungicide (conc.)[1]	Incidence of seedling damping-off(%)			
	AG 1[2]	AG 2-2	AG 4	AG 5
hymexazol (40 ppm)	72.4	30.9	100.0	88.4
tolclofos-methyl (40 ppm)	6.1	1.0	1.1	1.1
PCNB (40 ppm)	79.4	27.8	46.7	19.7
control 0	87.3	55.6	98.3	84.8

1) concentration in paper pot soil

2) AGs were inoculated to soil in paper pot

developed in 1957, its use has been increased year by year and now more than 95% of beet growers are using this method in Hokkaido. The method was developed for the purpose of extending the vegetation period and increasing the sugar yield, it also proved to be an excellent and promising method in disease control. One example is its effectiveness in the control of northern root-knot nematode (*Meloidogyne hapla*) once a serious problem in several localities in Hokkaido. The nematode is now scarcely found, because infestation has been prevented in paper pots filled with nematode-free soil. Also damping-off due to either *Pythium* spp. or *Aphanomyces cochlioides* is now so serious as in direct sown sugar beet, if the soil moisture in paper pots is adequately controlled.

The main principle in preventing damping-off in paper pots is to use the pathogen-free or sterilized soil. However, when the paper pots are filled with steamed or fumigated soil with a pH below 5.5, *Trichoderma* sp. vigorously proliferates utilizing cellulose of pot walls, and causes severe seedling blight[6], The adjustment of soil acidity is not recommended, because infection of *Polymyxa betae*, the vector of BNYVV (Rhizomania virus, a virus causing extreme decrease in sugar yield) is suppressed in soil below pH 5.5. From these points chemical control is an inevitable practice in the growth of sugar beet seedlings.

The registered fungicides in soil treatment of paper pots are PCNB for *R. solani*, hymexazole (Tachigaren for *Pythium* spp. and *Aphanomyces cochlioides*. Formerly, they were mixed with soil in advance, but now they were applied in the surface layer of paper pot soil, as Tokkiban (teat plate) was used in seeding operation as shown in the following figure. Thus, the amount of funicides applied is reduced: 50g of PCNB (5% dust) and hymexazole (4% dust) are uniformly mixed with 10 *l* of sieved soil for 8,400 paper pots for a field of 10 a. After seed germination, drench of soil with hymexazol solution is recommended.

In the test of anti-Rhizoctonia fungicides, there are several chemicals to

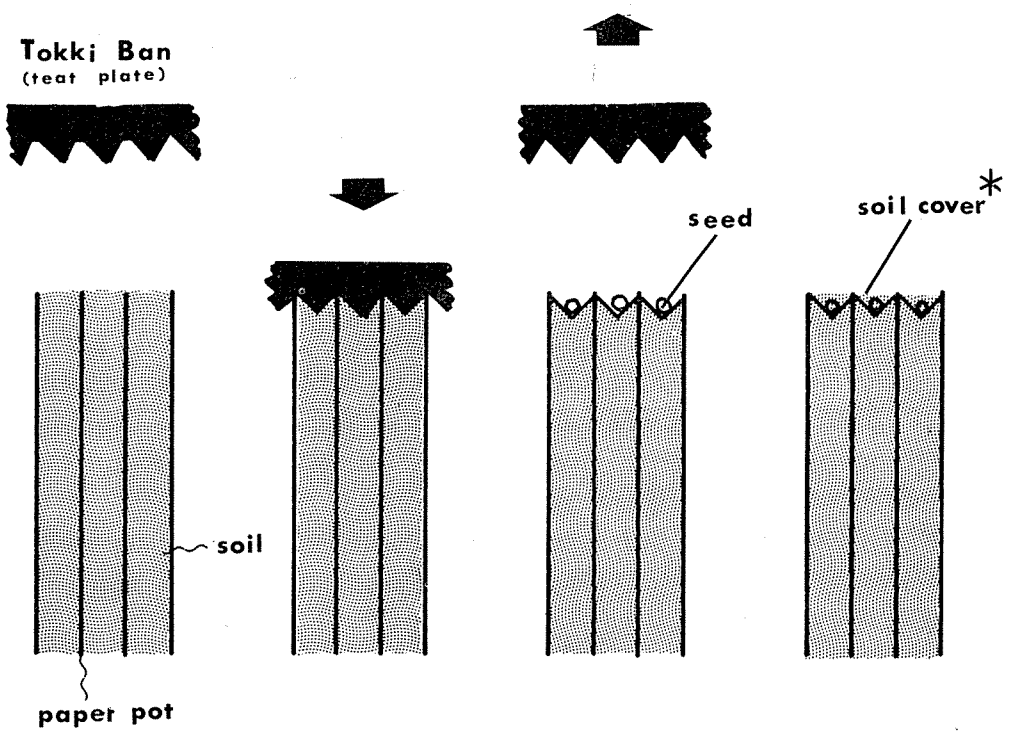

Fig. 3. Application of fungicides into the soil cover of paper pot. Soil cover, mixed with fungicides PCNB (5% dust) 50g and hymexazol (4% dust) 50g were mixed with 10 *l*. of sieved soil. PCNB will be replaced by tolclofos-methyl.

Table 3. Control of root rot by fungicide spray[1)] (source: Ass. Hokkaido Sugar Beet Tech. 1982)

Fungicides[2)]	Root rot (index)[1)]			Root yield (t/ha)
	July 14	Aug. 12	Sept. 10	
PCNB (X 500)	0.19	0.69	1.01	58.3
mepronil (X 500)	0.22	1.25	1.95	48.4
tolclofos-methyl (X 1000)	0.16	0.42	1.18	56.5
control (0)	0.90	3.69	4.12	10.9

1) The test in the field: paper pot grown sugar beet seedlings were transplanted at the end of April, and inoculated with AG2-2 June 25, 1982.

2) fungicides solution was sprayed to crown from both sides of the plants. Date of spray: June 30, July 14. Dosage: 200 *l*/10a
PCNB: 75% wetable powder, mepronil (Basitac): 75% W. P., tolclofos methyl (Rizolex): 50% W. P.

replace PCNB which has been found to be slightly phytotoxic to beet seedlnigs. They are mepronil (Basitac) and tolclofos-methyl (Rhizolex). Both are expected to be registered soon.

After the pot grown seedlings are transplanted, spray of funicides, such as PCNB, TPN or mepronil to the crowns is recommended after the middle of July. The spraying should be done before the fungus starts to infect petioles and crowns. Mepronil and tolclofos-methyl also give good control (Table 3).

Control of foliage blight is achieved by the spray of fungicides. Registered ones are mepronil (Basitac) TPN, TPTH, and iprodioine (Rovral), among them mepronil and iprodione are most effective.

In conclusion, the control of diseases by fungicides, together with the increased application of fertilzers, and wide spread of the paper pot transplanting method greatly contribute to the remarkable increase in the yield of sugar beet root and sugar in Hokkaido (Fig. 4).

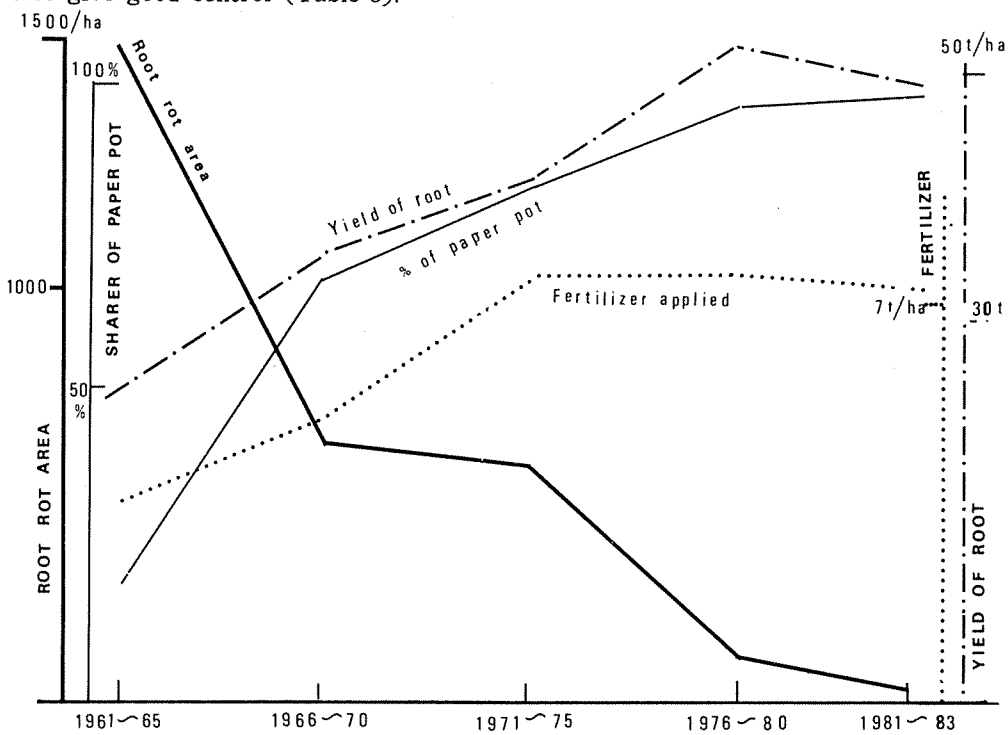

Fig 4. Decreaseing Rhizoctonia root rot and increasing the production of sugar beet by using fungicides, fertilizers and paper pot transplanting method during 1961~1983 in Japan (source; Nippon Beet Sugar Manufacturing Co.).

Prospects of biological control

There have been a number of field trials conducted to control sugar beet root rot by the incorporation of various materials into soil, however, the results have been inconsistant and few of the added materials are suitable for use.

In our experiments, severe root rot resulted from inoculation with AG 2-2 disappeared in the following year in the plants grown in the same plots: disease declined after the initial severe outbreak of root rot.

The decline was found associated with marked reduction of germinable sclerotia, suppression of hyphal growth, and hyphal lysis in soil. Rodshaped bacteria were observed on scleortia and hyphae. Also, diseased isolates of *R. solani*[4] which showed abnormally slow growth in early stage of colony development on agar plate, were obtained from the soil. All of these are believed to be contributing to reduction of inoculum potential of *R. solani* in soil[3].

Reinoculation with AG 2-2 in the following year to the declined plots caused little root rot: after decline, the soil became suppressive to the disease (Table 4). Heat treatemnt of the soil at 100°C for 30 min. eliminated suppressiveness of soil, at 80°C diminished suppressiveness was reduced to a lower level and at 60°C the treatment did not produce any effect. Also, drench of soil

Table 4. Reinoculation of declined soil with *R. solani* in fields receiving inoculation with same fungus previous year (in Experimental Farm in Obihiro[1])

Year of inoculation			Root rot (index) in		
1980	1981	1982	1980	1981	1982
R	R	R	4.28	1.38	2.31
R	O	O	4.38	1.10	0.05
O	R	O	0.10	3.94	0.71
O	O	R	0.09	0.01	3.63

R: inoculated with *R. solani* AG2-2 in the middle of June
O: not inoculated
— root rot index in the year of inoculation
1) sugar beet was monocultured during the experiments

Table 5. Suppressive effect of declined soil in paper pots[1]

Soil in paper pot	Inoculation after transplanting[2]	Root rot index				Yield[3]
		19/VI	16/VII	13/VIII	9/IX	
declined soil	−	0.00	0.01	0.01	0.01	59.3
	+	0.00	1.54	3.62	3.98	20.4
non-declined soil	−	0.00	0.00	0.00	0.08	57.6
	+	0.00	2.23	4.36	4.57	9.0
						LSD 3.9 (1%)

1) Sugar beet was grown in the paper pot filled with declined or nondeclined soil and transplanted into a field and inoculated with AG 2-2.
2) Date of inoculation: June 25
3) t/ha

with streptmycin (2,000 ppm) diminished it partially. From these resullts, soil sppressiveness is thought to be due to multiplication of nonspecific antagonistic microorganisms following the increased population of *R. solani*, especially of the sclerotia produced around severely diseased roots[2].

In the next experiment, sugar grown in paper pot filled with disease declined soil, and the plants were inoculated after they were transplanted into a field (Table 5). Root rot was slightly suppressed and yield of root was significantly high, but suppressive effect was not enough. Efforts are being made to increase the suppressiveness of paper pot soil for achieving improved disease control.

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Distribution and Ecology of *Pythium* Species in Japan

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摘　　要

Watanabe, T. (1985) 日本猝倒病菌 (***Pythium***) 之分布與生態 (日本農林水產省林業試驗本場)

本文所討論之日本產*Pythium* spp. 之分佈及生態是1968～1984年間由日本各地採取80種植株根部及 263 種土壤樣品分離所得之試驗結果。植株根部組織經洗淨後置於水瓊脂平板上，由長出之*Pythium* spp. 之菌絲尖端移植培養後供試。以修正過的Waksman之直接接種法，或是用胡瓜種子等供做土壤分離 *Pythium* spp. 用。分離時至少在二種不同溫度下進行，一是在 10°C 以下，另一是置於 20°C 以上，放置 1 至 7 天。試驗結果顯示與植物根部有密切關聯之優勢種類順序爲 *P. sylvaticum*, *P. spinosum*, *P. aphanidermatum* 和 *P. ultimum*，上述每一種類皆由 10 個以上之樣品分離而得。*Pythium* spp. 普遍存於日本國內土壤。在 263 個樣品中有 247 土樣 (佔 93.9%) 有 *Pythium* spp. 之存在，其中四種最常見者與根部密切關聯之優勢種相同。第五群之 *Pythium* spp. 僅形成類似菌絲之孢子囊及游走孢子，而沒有性器官，暫以 H-Zs (hyphae-zoospores) 名之，此群之分離出現頻率順序爲 *P. irregulare*, *P. vexans*, *P. splendens* 及 *P. torulosum*。優勢種因不同地區而稍有差異，但一般年平均溫高的地方 *P. aphanidermatum* 出現較多。所有供試之每一土樣可分離到 2 至 3.4 種 *Pythium* spp., 平均爲 2.6 種。以修正過的 Waksman 直接接種法分離 *Pythium* spp. 之量發現，由 Tohoku 及琉球羣島採取之 48 種土樣，其中之 12、17 及 19 種土樣每克土壤中可分得 201 以上，51～100 及少於 50 之 *Pythium* spp. 繁殖體。

ABSTRACT

Distribution and ecology of *Pythium* species in Japan were discussed on the basis of data obtained by assaying 80 plant root samples and 263 soil samples collected in various districts in Japan during 1968-1984. *Pythium* cultures were obtained from plant roots by placing washed tissue pieces on plain water agar plates and single hyphal tips from the fungal growth were

cut and transferred for further cultural works. Either a modification of Waksman's direct inoculation method or trapping with cucumber seed and other substrates was used to isolate *Pythium* spp. from soil. All isolation works were carried out at least at two temperature levels, with one below 10 C and one above 20 C. One to 7 days of incubation was allowed. The dominant species associated with plant roots were *P. sylvaticum*, *P. spinosum*, *P. aphanidermatum*, and *P. ultimum* in the descending order. These species were each isolated from more than 10 samples. *Pythium* species were ubiquitous in Japanese soils, being detected in 247 (93.9%) out of 263 samples. The four most dominant species were same as those from plant roots. A fifth group of *Pythium* which formed only hypha-like sporangia and zoospores, but had no sexual organs, was tentatively named H-Zs, an abbreviation for hyphae-zoospores. This was followed by *P. irregulare*, *P. vexans*, *P. splendens*, and *P. torulosum* in frequency of isolation. The dominant species was somewhat different with districts. *Pythium aphanidermatum* was more frequently detected in districts with a high yearly average of air temperature. Based on total soil samples the average yield was 2.6 species per sample while the average yield ranged from 2 to 3.4 species per soil sample, depending on districts. In assaying population of *Pythium* spp. by a modification of Waksman's direct inoculation method, 12, 17, and 19 out of 48 soil samples from Tohoku and the Ryukyu Islands yielded a total number of propagules exceeding 201; in the range of 51-200 and fewer than 50 per g. of soil, respectively.

INTRODUCTION

Although more than 200 literature have been published on *Pythium* diseases of various plants in Japan, only a few reports dealt with the distribution and ecology of *Pythium* species. In the course of an etiological study of soilborne diseases of higher plants in Japan, data on *Pythium* spp. as well as on diseases they incited have been accumulated. I shall discuss the subject on the basis of these data.

Identification of isolates.

Of the nearly 4,000 isolates studied some were difficult to identify, because they did not form sexual organs and showed no other distinctive morphological characteristics, except spherical or filamentous sporangia. Zoospores were never produced by isolates with spherical sporangia. A second group of fungi was provisionally named H-Zs, an abbreviation for hyphae-zoospores, because they formed zoospores from hypha-like sporangia. The zoospore-nonproducer isolates were tentatively identified as *P. sylvaticum* Campbell et Hendrix[2]. They included those that were definitely *P. sylvaticum*. The H-Zs isolates could well be *P. flevoense* Van Der Plaats-Niterink[14], a heterothallic species with hypha-like sporangia.

Table 1 lists the 31 species of *Pythium*, including H-Zs, recorded from the this study, together with the representative isolates.

Table 1. *Pythium* species and the representative isolates studied

Oogonia (echinulate)

1. *P. acanthicum*: 80-228 (Cabbage field, Kagoshima); 81-256 (Eggplant field, Fukushima)
2. *P. echinulatum*: 822 (Strawberry, Saitama, ATCC 36437): 81-219 (Radish field, Kanagawa)
3. *P. irregulare*; 78-2452 (Peanut field, Aomori) 81-30 (Cabbage field, Kanagawa)
4. *P. periplocum*(?): 78-2243 (Watermelon field, Aomori)
5. *P. spinosum*: 160 (Strawberry, Shizuoka, ATCC 36438); 79-468 (Eggplant field, Kagoshima)

Oogonia (smooth)

A. Hypha-like sporangia

6. *P. angustatum*: 257 (Strawberry, Shizuoka, ATCC 36485)
7. *P. apleroticum*: 841 (Strawberry, Saitama, ATCC 36441)
8. *P. dissotocum*: 81-708 (Lettuce field, Chiba)
9. *P. monospermum*: 80-571 (Pea field, Kagoshima)
10. *P. sulcatum*: 83-160 (Carrot, Chiba, Nagai's isolate)

B. Lobulate sporangia

11. *P. aphanidermatum*: 54 (Strawberry field, Shizuoka, ATCC 36431): 79-71 (Cucumber, Okinawa)
12. *P. deliense*: 79-76 (Pumpkin, Okinawa); 79-226 (Adzuki bean field, Kagoshima)
13. *P. graminicolum*: 80-214 (Soybean field, Miyazaki); 80-302 (Corn field, Kumamoto)
14. *P. inflatum*: 51 (Strawberry field, Shizuoka, ATCC 36436)
15. *P. myriotylum*: 864-1 (Strawberry, Saitama ATCC 36440); 83-193 (Chinese arrowhead, Saitama)
16. *P. torulosum*: 256 (Strawberry, Shizuoka, ATCC 36484); 81-327 (Grassland, Hokkaido)
17. *P. zingiberum*: 82-569 (Ginger, Wakayama); 82-724 (Ginger field, Wakayama)

C. Spherical sporangia

18. *P. nelumbium*: 84-209 (Bamboo field, Kohchi)
19. *P. oedochilum*: 165 (Strawberry, Shizuoka, ATCC 36433); 82-634 (Spinach, Nara)
20. *P. paroecandrum*: 827 (Srawberry, Saitama, ATCC 36432); 83-400 (Forest soil, Shizuoka)
21. *P. pulchrum*: 79-33 (Watermelon field, Aomori); 81-386 (Corn field, Hokkaido)
22. *P. rostratum*: 81-424 (Potato field, Hokkaido): 82-143 (Melon field, Chiba)
23. *P. splendens*: 192 (Strawberry, Shizuoka, ATCC 36444); 82-17 (Melon, Chiba)
24. *P. sylvaticum*: 866 (Strawbesry, Saitama, ATCC 36442); 80-164 (Watermelon field, Fukuoka)
25. *P. ultimum*: 901 (Strawberry, Saitama, ATCC 36443); 80-225 (Sweet potato field, Kagoshima)
26. *P. vexans*: 79-203 (Chinese cabbage field, Kagoshima); 80-274 (Cucumber field, Fukuoka)

No. sexual organ formed

27. *P. afertile*: 663 (Strawberry, Shizuoka, ATCC 36439)
28. *P. carolinianum*: 5 (Strawberry field, Shizuoka, ATCC 36434); 79-170 (Cabbage field Okinawa)
29. *P. elongatum*: 79-72 (Tomato, Obinawa)
30. *P. intermedium*: 483-2 (Strawberry field, Shimane ATCC 36445); 81-395 (Forest soil, Hokkaido)
31. H-Zs: 81-402 (Corn field, Hokkaido); 82-692 (Radish field, Osaka)

Pythium species associated with higher plants.

For isolation of *Pythium* from plant roots, tissue pieces (less than 5 mm long) dissected from random root samples were washed in running tap water for 0.5-1 h, blotted, and placed on water agar plates without previous surface sterilization. Hyphal tips were selected at random from the fungal growth from each tissue piece after 1-7 day incubation at two temperature levels, one below 10 C and one above 20 C, and were transferred to PDA plates for further cultural works.

A total of 682 *Pythium* cultures were obtained by assaying 80 root samples from 35 higher plants collected in various districts in Japan during 1968-1984. They were classified into at least 24 species, and the most dominant species were *P. sylvaticum*. *P. spinosum*, *P. aphanidermatum* and *P. ultimum* in the descending order (Table 2). They were each

Table 2. Detection and isolation of *Pythium* spp. from plant roots in Japan

Location (District)	Host	Sample no.	Isolate no.	No. of samples with Pythium[1)]				
				Pa	Pspi	Psyl	Pu	Others
Hokkaido	Azzuki bean, Bean	2	6	0	0	2	0	2
Tohoku	Cucumber and 7 others	8	43	4	2	4	2	4
Kanto	Strawberries and 10 others	24	280	4	5	5	1	15
Chubu	Strawberries and 6 others	12	109	0	6	5	2	8
Kinki	Strawberries and 6 others	11	111	1	3	3	4	7
Chugoku	Ginger and 4 others	4	28	0	1	1	1	4
Shikoku	Tomato and 3 others	3	4	0	1	1	0	1
Kyushu	Cucumber and 11 others	16	101	8	2	2	2	7
Total		80	682	17	20	23	12	48

1) Pa=*P. aphanidermatum*, Pspi=*P. spinosum*, Psyl=*P. sylvaticum*
Pu=*P. ultimum*, Others=P. deliense and miscellaneous species.

detected and isolated from more than 10 samples. These species had a wide host range[8], and several *Pythium* spp. could be detected from a single plant species. For example, 14 *Pythium* species were found associated with strawberry roots that were examined for the purpose of determining the etiology of a new stunt disease[24]. Data were summarized in Table 3. In the second example,

Table 3. *Pythium* species and number of isolates from strawberry roots and the rhizosphere soils in Japan Watanabe et al.(1977)

	No. of isolates			No. of isolates	
	Roots	Soils		Roots	Soils
P. afertile	2	—	*P. myriotylum*	2	—
P. angustatum	4	1	*P. oedochilum*	40	—
P. aphanidermatum	—	16	*P. paroecandrum*	3	—
P. apleroticum	1	—	*P. spinosum*	42	14
P. artotrogus (?)	5	6	*P. splendens*	3	9
P. carolinianum	—	49	*P. sylvaticum*	138	12
P. echinulatum	1	2	*P. torulosum*	2	—
P. inflatum (?)	1	—	*P. ultimum*	46	1
P. intermedium	—	1	*Pythium* sp.	1	—
TOTAL				291	111

diseased cucumber plants collected at Yamagata in 1978 were found to harbor *P. aphanidermatum* and four other species, but several samples collected in other areas yielded only *P. aphanidermatum*[20]. In another example, *P. splendens* was most frequently isolated from roots of wilted melon in vinyl houses in Chiba prefecture[25], but *P. irregulare* and *P. spinosum* were very rarely isolated from these materials. Samples yielding the latter fungi were from different locations at different sampling dates.

Ubiquitous distribution in soils.

Although nearly 40 species of *Pythium* were isolated and identified from higher plants, algae, and water before 1970, there has been little work on their isolation directly from soil. Therefore, the isolation of *Pythium* from the strawberry rhizosphere soils may be significant (Table 3).

Isolation from soils was conducted either by a modification of Waksman's direct inoculation method or by trapping. In the former case, less than 2 mg of soil samples was placed on plain water agar plates, and after incubation for 24-48 hr, pure isolates were obtained by single hyphal tippings from suspected *Pythium* colonies.

In trapping, soil samples weighing 10 to 50 g were mixed with trapping substrates. Sufficient water was added to keep petri dishes in a flooded condition. Potato discs, 4 mm in diam, were used as trapping substrates before 1978 and cucumber seeds were used after 1978. Following an incubation period of 1-7 days at a temperture below 10 C or above 20 C, the substrates were removed, washed under running tap water over 30 min., blotted to remove surface water, and placed on plain water agar plates. Further procedures were same as in the Waksman's direct inoculation method.

A total of 263 soil samples were collected from farmlands rice paddy

Table 4. Detection and isolation of *Pythium* spp. from soils in various districts of Japan

Location (District)	1968—1977				1978—1984			
	No. of samples		Rate of detection %	No. of isolates obtained	No. of samples		Rate of detection %	No. of isolates obtained
	Tested	Detected			Tested	Detected		
Hokkaido	7	7	100	65	21	20	95.2	250
Tohoku	—	—	—	—	28	26	92.9	221
Kanto	10	10	100	833	21	20	95.2	276
Chubu	57	54	94.7	386	12	8	66.7	111
Kinki	1	1	100	2	28	26	92.9	238
Chugoku	6	3	50	6	—	—	—	—
Shikoku	—	—	—	—	26	26	100	237
Kyushu	—	—	—	—	36	46	100	619
Total	81	75	92.6	1292	182	172	94.5	1952

fields, grasslands or forests in various districts in Japan in more than 40 occasions since 1968. Some of the 81 samples collected before 1978 were assayed only by the direct inoculation method, while trappind with cucumber seeds was used exclusively since 1978. *Pythium* species were detected in nearly 94% of the 263 soil samples assayed (Table 4). Sixteen samples yielding no *Pythium* propagules might be free from the fungi or otherwise the fungal populations must have been too low to be detected. However, it is certain that *Pythium* species could be detected in most soils in any district of Japan by the trapping method using cucumber seed or other substrates.

Dominant species in soils and number of species per sample.

At least 25 species of *Pythium* were detected or isolated from soil from all over Japan. The number of detected species varied with districts, ranging from 10 species in Hokkaido to 17 in Kyushu. *Pythium sylvaticum* was the most widely distributed species, being detected in 125 out of 182 soil samples collected in 1978 to 1984. Other dominant species were *P. spinosum*, *P. aphanidermatum* and *P. ultimum* in the descending order. If the sample number of fungus detection to the total number of samples assayed is defined as detection rate, that of *P. sylvaticum* was 68.7% and those for the latter 3 species ranged from 24 to 27%. The dominant species in different soils are illustrated, together with the detection rates in Fig. 1.

The number of species present in a soil sample differed with samples. Of 182 samples assayed an average of 2.6 species per sample was obtained (Fig. 2). However, as many as 6 species were detected per sample in 5 samples. By district, the average yield was two to 3.4 species per sample.

Pythium sylvaticum was usually the most frequently detected from each district. Other dominant species were encountered less consistently, For example, the dominat species were *P. sylvaticum*, *P. aphanidermatum*, and *P.*

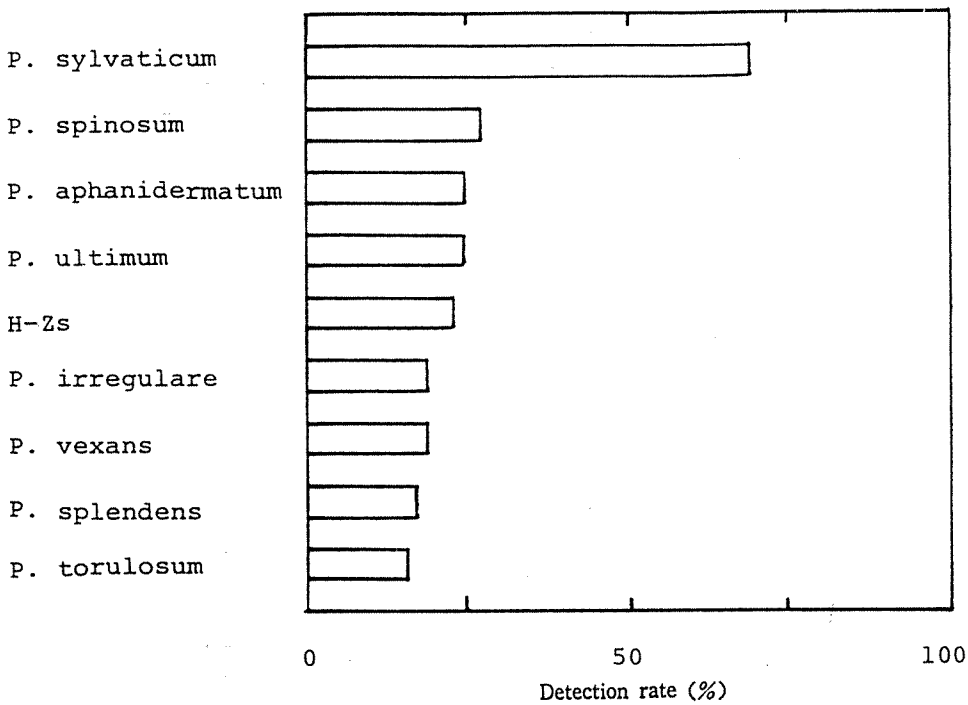

Fig. 1. Dominant *Pythium* species found in Japanese soils and their detection rates. Samples of 1978-1984.

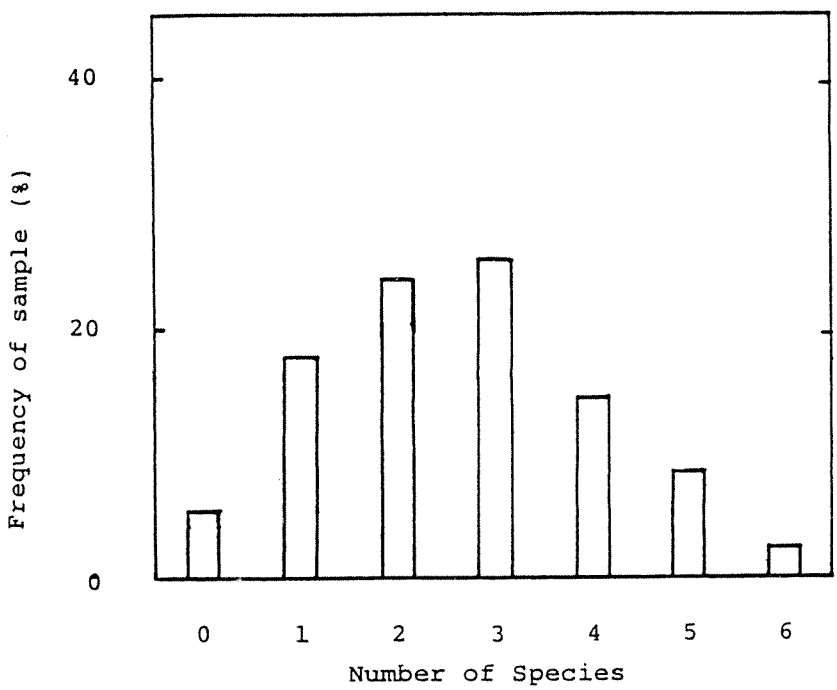

Fig. 2. Frequency (%) of soil samples with indicated numbers of *Pythium* species in Japan

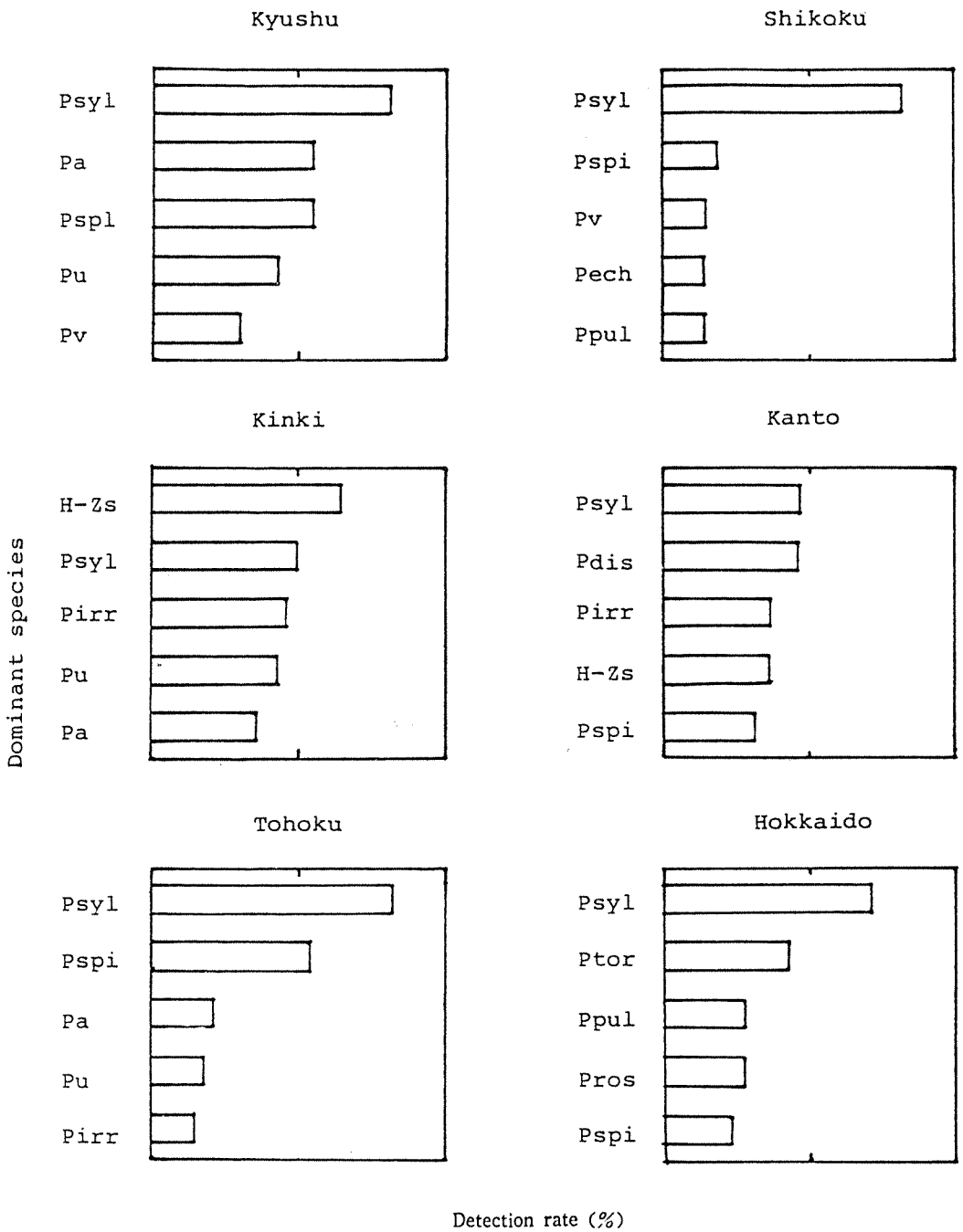

Fig. 3. Dominant *Pythium* species found in soils from six districts of Japan and their detection rates. Samples of 1978-1984. Pa=*P. aphanidermatum*, Pdis =*P. dissotocum*, Pech=*P. echinulatum*, Pirr=*P. irregulare*, Ppul=*P. pulchrum*, Pros=*P. rostratum*, Pspi=*P. spinosum*, Pspl=*P. splendens*, Psyl =*P. sylvaticum*, Ptor=*P. torulosum*, Pu=*P. ultimum*, Pv=*P. vexans*, H-ZS=a group of fungi with hypha-like sporangia, but without sexual organs.

Table 5. Incidence of soil-borne diseases of cucumber seedlings[1] in relation to populations of *Pythium* spp. in soils collected in six prefectures in southern and northern Japan

Based on Watanabe (1981, 1983)

Location (Prefecture)	Population (Propagules/g soil)	Sample no.	Disease incidence Average (%)
Okinawa	216.6[a]	11	94.5[ac]
Kagoshima (Amami-Ohshima)	62.1[a]	10	56.8[b]
Akita	215.9[a]	8	70.8[bc]
Aomori	123.8[a]	10	57.4[b]
Iwate	72.7[a]	5	69.4[bc]
Yamagata	20·2[a]	4	65 [bc]

1) No. of collapsed seedlings (no. of ungerminated seeds included)/no. seeds sown x 100. Numbers followed by the same letter do not differ significantly (P=0.01).

Table 6. Populations of *Pythium* spp. in soils

Location	Habitat and sample no.	Population	Literature
ENGLAND			
Kennington	Forest nursery (4)	55-687/g	Warcup (1952)[16]
Australia			
Southern Aust.	Forest nursery (18)	24-226/cm^3	Vaartaja & Bumbieris (1964)[13]
Adelaide	Pea, uncultivated land (4)	16-343/g	Kerr (1963)[6]
CANADA			
Ontario	Forest nursery (34)	0-92.5/cm^3	Vaartaja (1968)[12]
U. S. A.			
Southeastern US	Forest nursery (70)	0-179/g	Hendrix & Campbell (1968)[3]
California	Arable land (?)	64-3814/g	Stanghellini & Hancock (1970)[11]
Arizona	Arable land (4)	10-250/g	Burr & Stanghellini (1975)[1]
Maryland	Peach orchard (?)	64-378/g	Mircetich & Fogle (1969)[9]
Maryland	Arable land (5)	0-558/g	Lumsden et al. (1975)[7]
JAPAN			
Ryukyu Islands	Arable land (21)	0-1040/g	Watanabe (1981)[20]
Tohoku	Arable & uncultivated land (27)	0-441/g	

splendens in Kyushu, H-Zs and *P. sylvaticum* in Kinki, and *P. sylvaticum* and *P. spinosum* in Tohoku. In Shikoku and Hokkaido districts, *P. sylvaticum* was again dominant (Fig. 3).

Distribution in relation to temperature responses.

Pythium aphanidermatum, *P. deliense* and *P. myriotylum* need high temperatures, 34-37 C, for the optimum vegetative growth[8,9]. They have been thought to occur only in warm areas. However, in this study, *P. aphanidermatum* was found present in 52.4% of the samples from the Ryukyu Islands, where the average air temperature is above 20 C, and in 48% of the test samples from Kyushu where the average air temperature ranges from 14 to 17 C. In the Tohoku district where the yearly average of air temperature is 10 C, the fungus was detected in 4 out of 27 samples assayed. No fungus was detected in 21 samples from Hokkaido where average air temperature goes below 8 C. Therefore, the detection rates of *P. aphanidermatum* increased with the increase in average annual air temperature for districts[22] (Fig. 4).

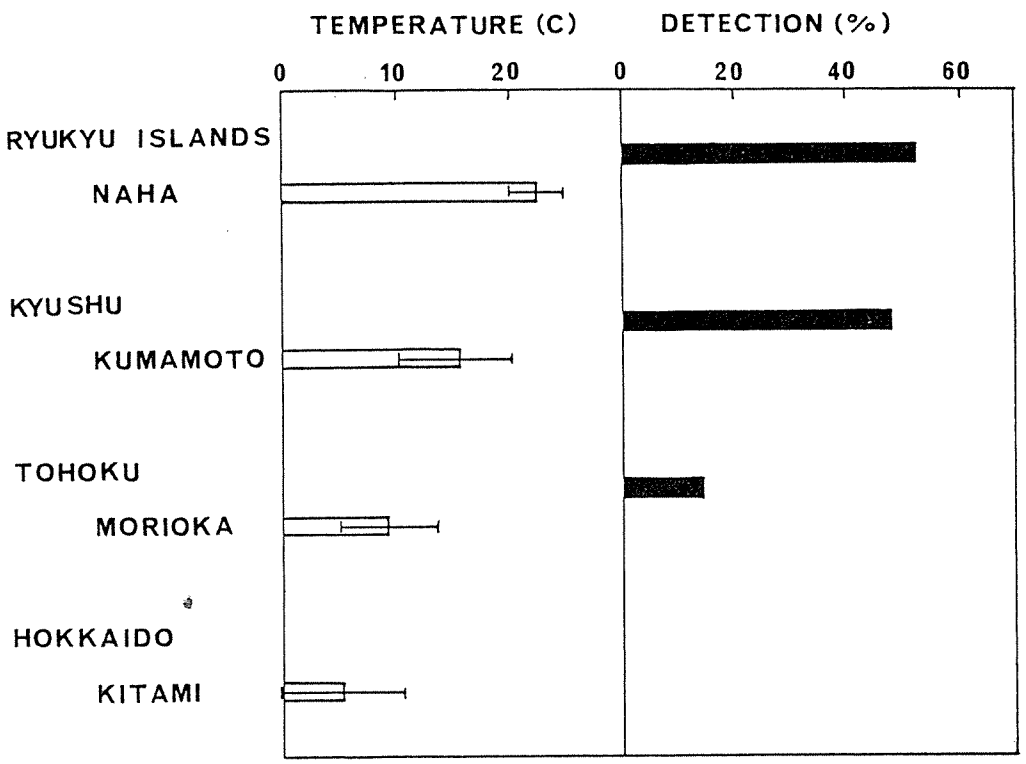

Fig. 4. Detection rates (%) of *Pythium aphanidermatum* in soil samples from the Ryukyu Islands and Kyushu in southern Japan and from Tohoku and Hokkaido districts in northern Japan in relation to the average annual air temperature in each area. Each thick bar represents the average annual air temperature with the maximum and minimum temperatures indicated by the solid line. Watanabe (1983)

The distribution of *P. deliense* appears to be limited to the Ryukyu Islands, and this fungus was isolated from pumpkin, tomato and bean roots, and also from five soil samples[21]. *Pythium. myriotylum* was isolated from strawberry[18,24] dasheen [10] and Chinese arrowhead[26] in Kanto district, but noteworthily it was also found on beans and in soil at Kitami of Hokkaido district[5].

In contrast to other 15 species from strawberry roots and the rhizosphere soils which had an optimum temperature at or above 28 C, *P. intermedium* grew best vegetatively at 25 C[19]. This fungus was detected in soils from various districts including Hokkaido and Mt, Fuji, especially by trapping made at temperature below 10 C.

Fungal populations and disease incidence in cucumber seedlings.

Only a few reports are available on the populations of *Pythium* in Japanese soils.

In this study, populations of *Pythium* spp. were determined in soil samples from Tohoku and the Ryukyu Islands, using a modification of Waksman's method[20]. Out of 27 samples from Tohoku assayed, nine samples each yielded more than 201 propagules per g of soil, 8 samples each yielded 51-200 propagules and 10 samples each yielded fewer than 50, whereas in soil samples from the Ryukyus, these propagule levels were found respectively in 3, 9 and 9 samples out of 21 tested. Dominant species composing these populations in Tohoku soils were *P. sylvaticum* and *P. spinosum*, and in the Ryukyu soils, it was *P. aphanidermatum*.

The highest propagule number was recorded in Okinawa soils, with an average of 1,039 per g of soil. All of the propagules belonged to *P. deliense*. Prefecturewise, Okinawa soils had an average of 216.6 per g of soil. The detail assay results are shown in Table 5, together with disease incidence data of cucumber seedlings. For comparison, similar data from the United States and other countries are given in Table 6.

We also determined disease incidence of cucumber seedlings as affected by *Pythium* populations in the soil samples in a previous work, Five seeds were sown in each of 50 g soil per dish with 30 ml of water, and incubated at 22 C. The collapsed plants were examined after an incubation period of 12 days.

The disease incidence differed statistically between Okinawa and Kagaoshima, and also between Okinawa and Aomori, but there was no significant difference when other prefectures were compared. Although pathogenicity test was not made in this study for each *Pythium* isolate. *P. aphanidermatum*, a pathogen of cucumber seedlings abundantly present in Okinawa soils, is believed to be closely related to the collapse of cucumber seedlings in Okinawa.

DISCUSSION

Pythium species were often found in roots of various plants, together with *Fusarium* and *Rhizoctonia* species in Japan [17]. Therefore, one tends to relate the *Pythium* isolates recovered to the soil-borne diseases observed, but this approach must be taken with caution, because the fungi are ubiquitous in soils and some of them may not be pathogenic.

The dominant species associated with roots were quite similar to those present in soils in Japan. Likewise, these fungi were also recorded as dominant in the soils of the continental United States[4].

Recently, a simple and reliable method was developed for detection and quantitative estimations of *P. aphanidermatum* in soil[23]. Further improvement is needed for better under standing of the distribution and ecology of *Pythium* species in soil and for taxonomic clarification of those ambiguous species in the group.

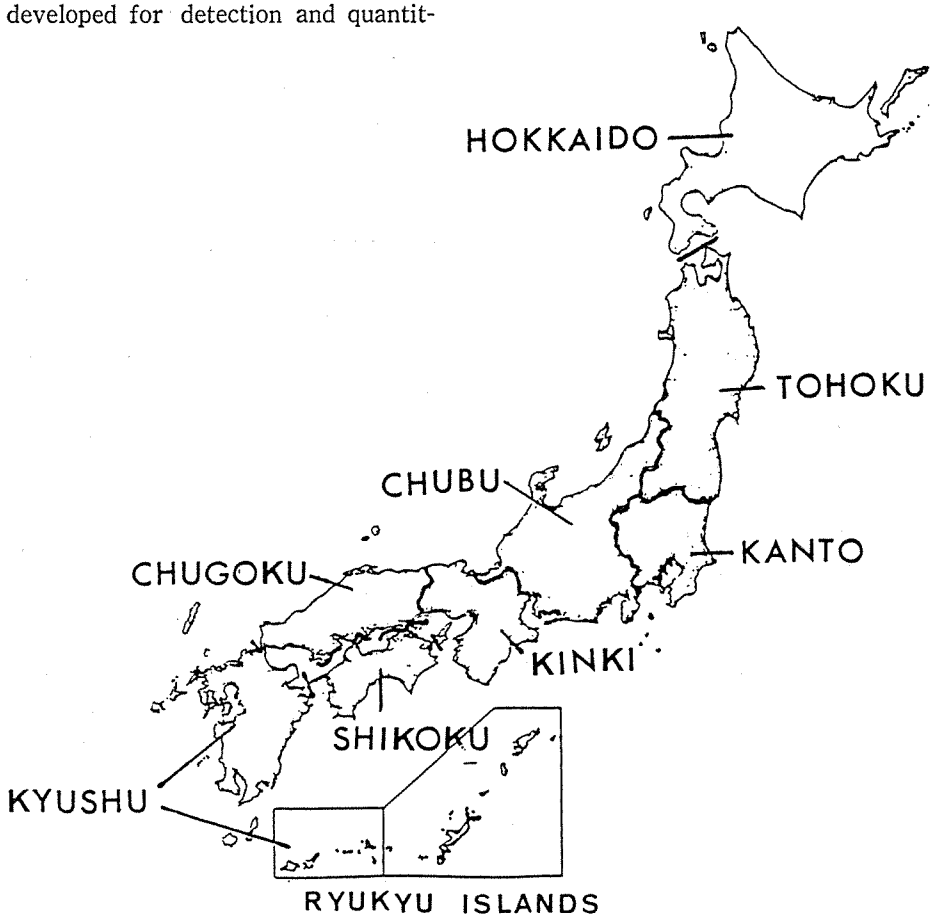

Fig. 5. Map of Japan

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Effect of Soil Amendment on Clubroot of Crucifers

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摘　　要

楊慶鴻、謝文瑞（1985）土壤添加物對十字花科蔬菜根瘤病之防治效果　（國立中興大學）

在土壤中添加 0.5%及 1 %的 S—H 混合物可有效抑制青江白菜根瘤病的發生，將 S—H 混合物之各成份以不同組合方式測試，發現蚵殼粉加礦灰及單獨使用礦灰皆具有幾乎與 S—H 混合物相同的防病效果。礦灰中含有 CaO、MgO、Al_2O_3、FeO 及 SiO_2 等成份，分別單獨使用前三者皆可降低青江白菜根瘤病根毛感染率。在田間試驗中，土壤添加礦灰、碳酸鈣及 S—H 混合物都可明顯抑制根瘤病的發生。由上述結果顯示土壤添加物中的鈣離子可能是防治根瘤病的最主要成份。

（關鍵字：十字花科蔬菜，根瘤病，土壤添加物，防治）

ABSTRACT

Amendment of soil with S-H mixture at 0.5 and 1% greatly reduced the incidence of clubroot disease on the cultivar Green-Petiole Pai-Tsai of *Brassica chinensis*. Among various combinations of the components of S-H mixture tested, oyster-shell powder plus mineral ash and mineral ash alone were almost as effective as S-H mixture in reducing clubroot disease. Mineral ash contained CaO, MgO, Al_2O_3, FeO and SiO_2. The first three, when applied singlely, greatly reduced the root hair infection of Green-Petiole Pai-Tsai. In the field, mineral ash, calcium carbonate and S-H mixture markedly reduced the incidence of clubroot disease. The above results suggest that calcium at the concentration present in the tested soil amendments is the most active component in controlling the disease.

(Key words: cruciferous vegetables, clubroot, soil amendments, control)

INTRODUCTION

Clubroot caused by *Plasmodiophora brassicae* Wor. has become serious in both the northern and central parts of Taiwan in recent years[8]. Mustard, Szechuan mustard, Pak-Choi, Chinese cabbage and rapeseed suffered considerable losses when grown in the infested field. The recommended control measures include liming of soil, the use of a wide range of chemicals and soil fumigation[1,3,4,5,17]. Although some soil sterilants and fungicides offer an adequate disease control, the values of the host crops do not usually justify adoption of a chemical means[1,17]. Lime appears to be effective in controlling clubroot when it is applied at a high rate. Its use is easy and inexpensive in comparison with other control chemicals[5]. However, the application of large quantities of lime in soil may often result in a reduced availability of some trace elements such as boron, iron and maganese[12]. Of the various methods commonly recommended for clubroot control[7], none is considered economical and has been used by farmers in Taiwan. We, therefore, investigated the possibility of controlling the disease with soil amendments.

MATERIALS AND METHODS

Test of soil amendments

The S-H mixture consisting of 4.4% bagasse, 8.4% rice husks, 4.25% oyster-shell powder, 1.01% potassium nitrate, 13.16% calcium superphosphate, 8.25% urea and 60.5% mineral ash has been found effective in preventing watermelon wilt caused by *Fusarium oxysporum* f. sp. *niveum* when mixed with infested soil at 1%[16]. This mixture was incorporated into the *Plasmodiophora brassicae* infested soil at 0.5% or 1% for test against clubroot disease. Each six Kg of sandy soil (pH 4.6) mixed with S-H mixture was placed in a plastic flat ($35 \times 27 \times 8.5$cm). To each flat was added 150 ml suspension of *P. brassicae* resting spores (4×10^7 spores/ml). Seeds of Green-Petiole Pai-Tsai of *Brassica chinensis* were sown in treated soil as well as in control soil. Data were collected after 37 days. The various components of the S-H mixture were also tested to determine if they are effective in reducing clubroot disease incidence.

A field trial was conducted to compare mineral ash and calcium carbonate with S-H mixture to control clubroot. The soil was sandy loam (pH 5.0). To an area of 1 m^2 was applied one Kg of S-H mixture, mineral ash or calcium carbonate. About 2,000 seeds of Green-Petiole Pai-Tsai were sown in each plot (8×4m). After thinning made one week later, 1,000 seedlings remained and 600 plants were examined for the presence of clubbed roots for each plot. The treatments were replicated five times and observations made after 48 days.

Test of mineral salts

Mineral ash contained in the S-H mixture had the following composition: SiO_2, 31%; CaO, 44%; MgO, 1.7%; Al_2O_3, 18% and FeO, 1%. The effects of the various components on infection of root hairs by *P. brassicae* were tested experimentally, $AlCl_3$, $CaCl_2$, $FeCl_3 \cdot 6\,H_2O$, $MgCl_2 \cdot 6\,H_2O$ and H_2SiO_3 were used at quantities so that the concentrations of each cation in the soil was corresponding to mineral ash applied at 0.5% and 1%.

Sixty grams of infested sandy loam soil at pH 5.6 was mixed with each of the test mineral salts separately and placed in a polyethylene container (8×6cm). Forty seeds were sown in each container. Four containers were used in each treatment. After one week, seedlings were gently removed from soil. Washed roots were immersed in 0.6% aceto-carmine solution [2] for two days, and then examined under a microscope for the presence of *P. brassicae* zoosporangia in root hairs. Four roots (2.5 cm long) were examined for each treatment.

RESULTS

Effect of soil amendment on clubroot

The infested soil amended with S-H mixture at 1% raised soil pH from 4.6 to 6.0. Amendment of soil with the mixture greatly reduced the incidence of clubroot disease (Table 1). None of the plants grown in soil amended with 1% S-H mixture had clubbed roots. Amendment of soil with S-H mixture at 0.5% also reduced the disease incidence from 34.6% in nonamended soil to 1.4% in amended soil.

Table 1. Effect of soil amendment with S-H mixture on incidence of clubroot on *Brassica chinensis* cultivar Green-Petiole Pai-Tsai

Amendment	No. of plants with clubbed roots/ No. of plants examined				Total	Plants infected (%)
	Rep. I	Rep. II	Rep. III	Rep. IV		
1% S-H mixture	0/47	0/44	0/47	0/29	0/167	0
0.5% S-H mixture	2/57	0/52	1/57	0/56	3/222	1.4
None	26/65	15/55	23/64	18/53	82/237	34.6

Table 2. Effect of components of S-H mixture on incidence of clubroot in *Brassica chinensis* cultivar Green-Petiole Pai-Tsai

Amendment	Conc. (%)	No. diseased plants/ No. plants examined	Plants infected (%)
S-H mixture	1	1/64	1.6 a[1]
Rice husks+urea+ oyster-shell powder	0.21	47/94	50.0 c
Urea+mineral ash	0.69	8/79	10.2 a
Oyster-shell powder+ mineral ash	0.65	1/163	0.6 a
Urea+calcium superphospate	0.22	17/62	27.4 b
Potassium nitrate+ calcium suphosphate	0.14	33/144	29.0 b
None		64/139	46.0 c

1) Within the column, values followed by the same letter do not differ significantly (P=0.05) according to Duncan's new multiple range test.

Among various combinations of the components of S-H mixture tested, oyster-shell powder plus mineral ash and urea plus mineral ash were almost as effective as S-H mixture in reducing disease incidence (Table 2). Since the combination of rice husks, urea and oyster-shell powder was not effective in suppressing clubroot, mineral ash appeared to be the major active component of S-H mixture.

In the field trial, calcium carbonate, mineral ash and S-H mixture were effective in reducing disease incidence of clubroot and increased the plant weight significantly (Table 3). At the rate of 10 t/ha, calcium carbonate, mineral ash and

Table 3. Efect of S-H mixture, mineral ash and calcium carbonate on incidence of clubroot on *Brassica chinensis* cultivar Green-Petiole Pai-Tsai in the field

Amendment[1)]	No. of diseased plants/No. of plants examined[3)]	Plants infected (%)	Plant weight (kg/3000 plants)
S-H mixture[2)]	638/3000	21.3 a[4)]	290.7 a
Mineral ash	605/3000	20.2 a	244.7 a
Calcium carbonate	435/3000	14.5 a	272.3 a
None	935/3000	31.4 b	184.4 b

1) About 1 kg of S-H mixture, mineral ash or calcium carbonate was incorporated into an 1 m^2 area.
2) S-H mixture minus urea.
3) Number of plants investigated in five replications in each treatment.
4) Means followed by the same letter in a column are not significantly different, P=0.05, by Duncan's multiple range test.

Table 4. Effect of mineral amendments on the root hair infection by *Plasmodiophora brassicae* on *Brassica chinensis* cultivar Green-Petiole Pai-Tsai

Cation (mineral)	Cation conc. added (ug/g)	No. of root hairs infected per plant[1)]	Soil pH
Al ($AlCl_3$)	150	0.3	4.9
	75	3.4	5.4
Ca ($CaCl_2$)	1000	0.9	5.5
	500	4.4	5.6
Fe ($FeCl_3 \cdot 6H_2O$)	100	108.7	5.4
	50	113.4	5.5
Mg ($MgCl_2 \cdot 6H_2O$)	200	10.6	5.7
	100	23.4	5.6
Si (H_2SiO_3)	2000	38.8	5.7
	1000	39.6	5.7
None		82.3	5.6

1) All values are mean of zoosporangia formed root hairs of 16 plantlets examined.

S-H mixture reduced the disease incidence from 31.4% to 14.5%, 20.2% and 21.3 %, respectively. Also the average plant weight of 3,0000 plants in 5 replicates increased by 47.7%, 32.7% and 57.7%, respectively.

Effect of mineral salts on root hair infection

There was no significant pH change of the soil when $AlCl_3$, $CaCl_2$, $FeCl_3.6\ H_2O$, $MgCl_2.6\ H_2O$, or H_2SiO_3 was added individually to the soil at the test concentration. The number of infected root hairs per root decreased from 82 in non-treated soil to less than 11 when soil was amended with 150 and 75 ppm of Al^{+++}, 1,000 and 500 ppm of Ca^{++}, or 200 ppm of Mg^{++}. However, 100 and 50 ppm of Fe^{+++} had no effect in reducing root hair infection (Table 4).

DISCUSSION

Application of mineral ash to soil, in combination with urea or oyster shell powder, was as effective as S-H mixture in controlling clubroot. This suggests that mineral ash was the active component of the mixture. Further supporting evidence came from the observation that deletion of mineral ash from S-H mixture rendered the latter ineffective in clubroot control, as seen in the greenhouse tests result with the combination of rice husks, urea and oyster shell powder (Table 2). The explanation is further supported by results which showed that mineral ash alone was as effective as S-H mixture in reducing root hair infection and clubroot in field trials.

Ko and Hora[11] reported that Al^{+++} at 0.65 ppm inhibited ascospore germination of *Neurospora tetrasperma*. Lewis[13] also showed that addition of Al^{+++}, Ca^{++}, Cu^{++} and Zn^{+++} at 50 and 100 ppm to soil significantly reduced root rot of peas caused by *Aphanomyces euteiches*. The mineral ash in our experiments consisted of SiO_2, CaO, MgO, Al_2O_3 and FeO. Among the cations, Al^{+++}, Ca^{++}, Mg^{++} and Si^{++++} appeared to be responsible for the suppression of root hair infection. However, because calcium carbonate alone at 1% was as effective as S-H mixture at the same concentration in reducing root hair infection and incidence of clubroot disease, we believe that Ca^{++} is the most important active component in mineral ash in controlling clubroot disease.

Liming the soil before planting has been the oldest practice used widely for the control of clubroot[10]. Using high rate of lime at 20 t/ha and raising soil pH up to 7.2 were effective in suppressing clubroot[5,10]. Carbonates of Na^+, K^+, Ca^{++}, Ba^{++}, $Mg^{++}$and and Al^{+++} raised soil pH from 6.6 to 7.2 and reduced the number of root hair infection by *Plasmodiophora brassicae*, while chloride of these cations did not raise soil pH and did not reduce the number of infected root hair considerably[15]. Our results showed that root hair infection decreased significantly when soil was amended with $CaCl_2$, $AlCl_3$, $MgCl_2.6\ H_2O$ or H_2SiO_3. However, soil pH did not change noticeably (Table 4). Fletcher et al reported[5] that soil amended with $CaSO_4$ (20 t/ha) reduced clubroot incidence, while the soil pH also was reduced from 7.0 to 6.6. Hamilton and Crête[6] reported that the increase in calcium ion concentration and in soil pH decreased the incidence of clubroot. At a low soil pH, calcium ion may be lost easily, resulting in Ca^{++} deficiency in the

soil. Continuous application of ammonium sulfate by Taiwan farmers in the past 30 years tends to decrease soil pH[14]. We suggest that application of mineral ash, calcium carbonate or S-H mixture at 1% will be an effective means in controlling clubroot of short-term cruciferous crops. Studies are underway to determine whether these soil amendments can also be effective in controlling clubroot disease of long-term crops, i.e. Chinese cabbage, mustard and Szechuan mustard.

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Ecology and Control of Fusarial Wilt of Banana

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摘　　要

黃新川（1985）香蕉黃葉病之生態與防治　（香蕉研究所）

目前臺灣香蕉發生黃葉病（病原菌：*Fusarium oxysporum* f. sp. *cubense* 生理小種 4）的蕉園面積每年達 1,500 公頃以上，發病株數於 1968年僅有 1,500株，到 1983年增至 500,000 株。本病病原菌普遍分佈於高、屏兩縣，局部性分佈於彰化、南投、和嘉義等縣。本病遠距離傳播主要以流水和病苗爲媒介，在蕉園內則可依賴灌溉水，除萌作業和種植病苗而擴散。溫度會影響本病的發生，將組織培養蕉苗接種後，在不同溫度條件下生長兩個半月後，調查發病率分別爲 17°C～10%，21°C～14%，25°C～17%，29°C～40%，32°C～58% 。傷害蕉株的根部會促進發病，在一田間試驗，在人爲傷根情況下接種後發病率爲67% ，但在根部沒有傷口之對照處理發病率僅有38%。從塊莖傷口接種則不能引起感染。病園土壤施加石灰沒有抑制發病的效果，但病園與水稻輪作後發病率呈明顯的下降，輪作水稻一年後發病率從原先之30～50%降至8.1～17.6%，輪作 2.5～3年後再降至 0.8～6.3% 。病園與旱作如甘蔗或向日葵輪作則無治病效果。病原菌主要分佈於病園土壤深度 20公分以內之表土層，在此深度以下則不能分離到病原菌。病園經輪作水稻一年之後，尚有 2%病菌族群密度殘存於深 3 公分以內的表土，但在 10 公分深以下其密度已降低到不能偵測的程度，輪作水稻 2 年後即不能從表土分離到殘存的病原菌。在抗病選種試驗中，從疫區蕉園遴選 3,000 餘株健康臺蕉測定，結果顯示都不具有適當程度的抗病性。以組織培養蕉苗做測定材料，第一年於抗病檢定園分批種植共計 13,878 株，得到 17 株沒有得病。

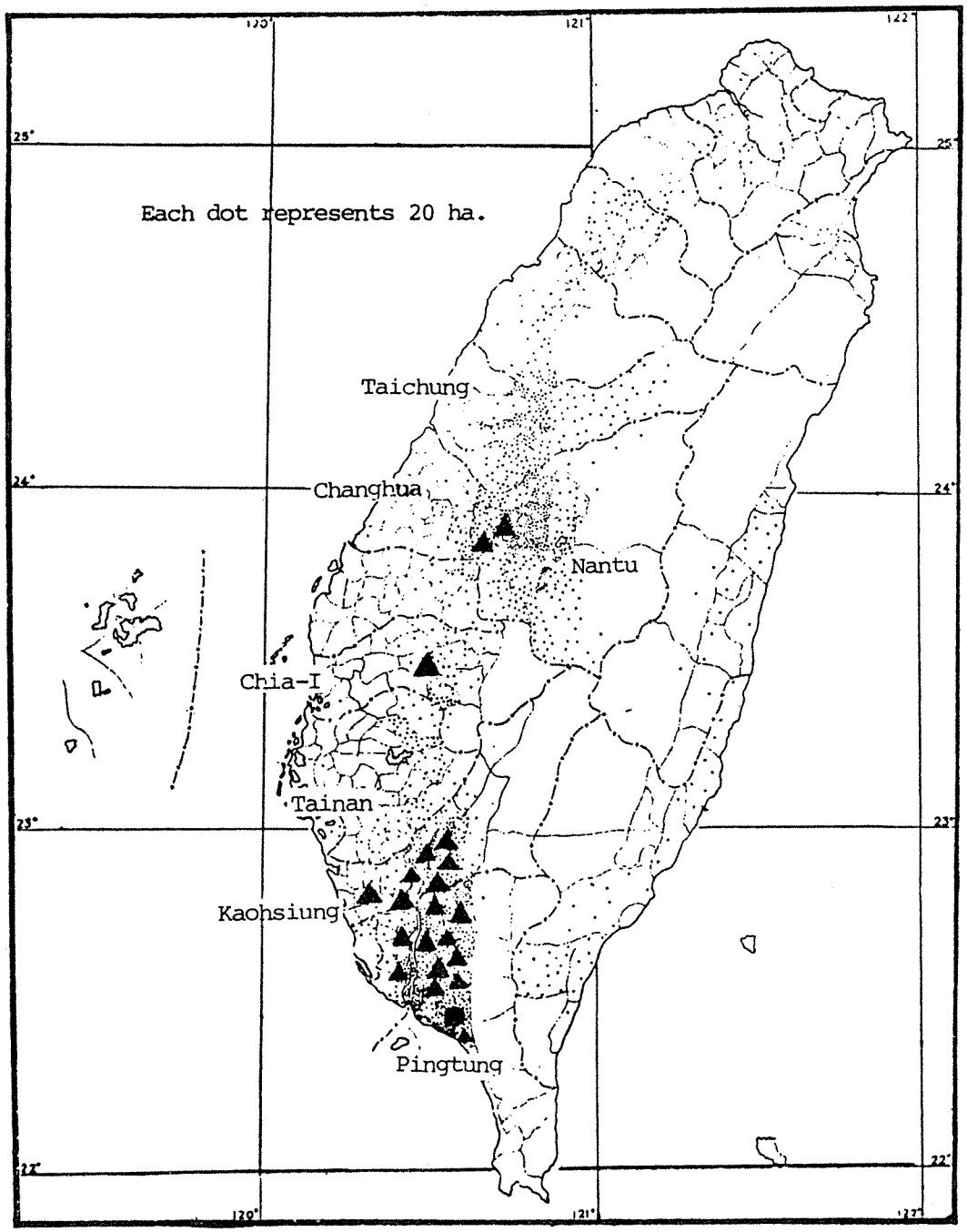
ABSTRACT

Fusarial wilt of banana (*Musa* AAA 'Giant Cavendish' and 'Robusta'). caused by *Fusarium oxysporum* f. sp. *cubense* race 4, is affecting more than 1,500 hectares of banana plantations annually in Taiwan. Cases per year increased from 1,500 in 1968 to more than 500,000 in 1983. The fungus was widely distributed in Pingtung and Kaohsiung counties, and also appeared

sporadically in Changhua, Nantu, and Chia-I counties. Long distance spread of the disease was mainly associated with movement of water and infected suckers, while disease spread within the field was mainly attributed to the irrigation water, pruning practice and planting of infected suckers. Disease incidence is affected by temperature. After 2.5 months of growth at various temperatures, percentages of infected banana plantlets were 10% at 17°C, 14% at 21°C, 17% at 25°C, 40 % at 29°C, and 58% at 32°C. Mechanical wounding of roots increased wilt incidence. In a field trial involving 60 plants tested, 67% of the plants became infected when inoculation was made under wounded conditions, as compared to 38% under unwounded conditions. Inoculation of wounded rhizome failed to establish infection. No reduction in disease was evident with soil liming. A substantial reduction in disease was found following rotation with rice. After one year in rice wilt incidence was reduced to 8.1-17.6% from a level of 30-50% originally, and after 2.5-3 years it was reduced further to 0.8-6.3%. Upland crops such as sugar cane and sunflower were not effective as a rotating crop. In the diseased orchard, the pathogen was distributed primarily in the upper 20 cm of surface soil, and was not detectable in soil beneath this depth. After one year in rice, 2% of the original fungal population remained surviving in the upper 3 cm surface soil, while it was not detected in soil below 10 cm in depth. After 2 years in rice, population of the fungus in surface soil was decreased further to nondetectable level. In a screen-for-resistance test conducted under field conditions for which more than 3,000 survivors had been selected from severely diseased orchards, none displayed any desirable level of disease resistance. In a mass screen program for testing banana plantlets derived from meristem culture under greenhouse or field conditions, 17 clones remained wilt-free from a total of 13,878 plantlets of Taiwan Cavendish screened in the first year.

INTRODUCTION

Fusarial wilt of banana, caused by *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder & Hansen, was the most devastating disease affecting commercial banana production in the Western hemisphere between 1900 and 1960. Over a period of 60 years, the disease had destroyed about 100,000 acres of banana plantations and influenced the economy of many countries[19]. Finally, it forced a change in banana cultivation from the susceptible Gros Michel to the resistant Cavendish varieties. Since the resistant Cavendish has been the major banana variety of world commerce, the disease is no longer a major problem in all banana growing countries, with exceptions of the Canary Island, Australia, South Africa, and Taiwan[11,20,21]. In Taiwan the disease, first noted in 1967, is most serious, and now it is affecting more than 1,500 hectares of banana plantations annually. The fungus that attacks Taiwan Cavendish was identified to be race 4 of *F. oxysporum* f. sp. *cubense*[2,21].



● Original infection centers noted in 1967.

▲ Areas with fusarial wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* race 4.

Fig. 1. The banana growing areas in Taiwan and distribution of fusarial wilt of banana.

With a banana trade of world commerce entirely based on Cavendish varieties today, appearance of the new race virulent to Cavendish varieties posts a threat to all banana growing countries. The outcome could be as disastrous as it once was in South and Central Americas if the disease is not contained. For the control of fusarial wilt in Taiwan, a great deal of effect has placed on the part of resistance selection, and its preliminary results were presented in this report. The spread of disease and the effect of such factors as temperature, soil liming, and mechanical wounding on wilt incidence were studied. Control through crop rotation was attempted.

OUTBREAK, SPREAD, AND DISSEMINATION

Of the approximately 8,000 hectares of banana grown in Taiwan, about 5,000 are grown for export in the South, and another 3,000 are grown mainly for local consumption in the more northern central district around Taichung (Fig. 1). The first cases of fusarial wilt on the Taiwan Cavendish cultivars were found in 1967 in Chiatong district of Pingtung county. The disease continued to spread but was confined around this area in the subsequent 5 years, the affected area increasing from 0.27 to 130 hectares in this period. In 1972, the disease appeared in Kaoshu, about 47 kilometers north of the original infection center.

In 1973, new infection centers were noted in Kaohsiung county. An emergency control measure was proposed for the first time and put into enforcement in the same year. Farmers were paid compensation to destroy diseased fields and to convert them into paddy rice. In 1978-1979, the disease was noted in Chia-I and Changhua counties in the more northern central part of Taiwan. The wilt pathogen attacking Taiwan Cavendish in these two areas also was race 4 of *F. oxysporum* f. sp. *cubense*[3,5]. It was found that the disease was spread from southern part of Taiwan to Chia-I via planting materials. But the route through which the disease was transmitted to Changhua, about 200 kilometers north of banana growing areas in the South, was not understood. At present, about 1,500 of the 5,000 hectares in southern Taiwan are affected. Within the 1,500 hectares about 10-15% of the plants are diseased. Cases per year had increased from 1,500 in 1968 to more than 500,000 in 1983.

Disease appearance is seasonal. The first cases were noticed in October and incidence increased from November through February the coldest month of the year. According to Su *et. al.*[21], the incubation period was longer than 6 months. Disease incidence is affected by temperature, When plantlets derived from meristem culture, 7-10 cm in height, grown in pot (9 cm high, 10 cm wide) containing soil with 500 propagules/g soil of wilt pathogen, were placed in growth chamber for 2.5 months at various temperatures, percentages of plantlets infected were 10% at 17°C, 14% at 21°C, 17% at 25°C, 40% at 29°C, and 58% at 32°C. Results indicate a higher temperature favors disease development, and in the field most infection occurs in the hot, rainy summer. In Central America, wilt incidence of Gros Michel was also greatest in summer months[19], and Rishbeth[14] mentioned that wilt was

less common in Jamaica above 2,000 feet during winter months when night temperature fell below 60°*F*.

Of the many ways by which *F. oxysporum* f. sp. *cubense* was disseminated, irrigation water and planting of infected suckers were the major factors responsible for long distance spread of disease in southern Taiwan. In the past, one of growers' common practices of 'eliminating' the pathogen from diseased field was to throw infected plants into the ditches or streams. Thus the pathogen in abundance was carried away by the water with subsequent outbreaks of disease along the movement of water. The fungus was frequently detectable in the stream water during the diseased season. In many instances, diseased plants were found in newly planted field as a result of planting infected suckers.

Once infected, disease spread within the field can be brought about by various ways. In some cases where the disease moved along the row, movement of irrigation water and the pruning practice were the major factors to account for this spread sequence. Since fragments of diseased tissues rich in the pathogen move easily in the irrigation ditches, disease can be spread rapidly from one plant to another along the row. Hwang and Su[4] provided evidence showing that the pathogen was carried by the pruning tool and caused infection from plant to plant as the pruning was done along the row. However, with annual replanting as a common practice in banana cultivation, the disease in most cases spread randomly in the field through the movement of infected suckers in subsequent replantings.

EFFECT OF MECHANICAL WOUNDING ON WILT INCIDENCE

It is not known how *F. oxysporum* f. sp. *cubense* initiates an infection. While some workers believed infection in the field was mainly via lateral roots[13,19,26], others believed entry occurred through wounded rhizome[1,27]. The mode of entry is also a controversial subject. For instance, Brandes[1] and Wellman[27] reported that infection occurred through uninjured roots; on the other hand, Salas [16] and Sequeira *et al*[17]. considered that mechanical wounding of mature main roots prior to inoculation was essential for infection.

Effect of root wounding on disease incidence···The experiment was carried out in a field free of disease. The suckers 120-150 cm tall from healthy mother plants were used as planting materials. Two months after planting, root inoculation was made by (A) placing inoculum in a small ditch (15 cm deep), dug 30 cm away around the rhizome, and (B) placing inoculum on the soil surface evenly distributed within 30 cm surrounding the rhizome. Many roots were cut off or damaged in the first treatment. Thirty plants were used for each treatment, and each plant was inoculated with about 900 g of chopped diseased tissues (7.5×10^5 propagules/g of diseased tissues). The inoculum was covered with soil after inoculation. About 67% of plants in the first treatment showed wilt symptom, while only 38% of plants were diseased in the second treatment after 9 months. Results showed that plants are more susceptible to fusarial wilt when their roots were

mechanically wounded. Wounding also increased the rate of disease development. The first case of wilt appeared about 3 months after inoculation under root wounding conditions, while it took about 5 months without root wounding.

Annual replanting is a common practice in banana plantation in southern Taiwan. A few months after planting the field is ploughed to make ridges for flood irrigation and drainage purpose. When the suckers are 4-5 feet tall, a bamboo prop is inserted post-like into the ground and tied to each plant. New suckers are dug out monthly up to the shooting stage. Fields are kept free from weeds by hoeing. These practices usually result in considerable root damage, and hence render the plants more susceptible to wilt. This may explain why fusarial wilt infection occurs more readily in replant suckers than in ratoon suckers, as was observed in the diseased areas.

Infection through rhizome···Three holes (3 cm deep) were excavated on each rhizome with a cork borer, and then were filled with diseased tissues. Inoculation was made either at time of planting or two months after planting, with 5 plants per treatment. None of 10 plants showed wilt symptoms by the end of harvest. In the greenhouse, banana plantlets derived from meristem culture [10], about 15 cm high, were inoculated in the same manner, and the result was also negative. Results of this study indicate that damage to the rhizome, such as tunnels made by weevil borers, is unlikely to provide entry for the wilt pathogen. It appears that in the field infection occurs exclusively from the roots.

EFFECT OF SOIL LIMING ON WILT INCIDENCE

Based on the rate of fusarial wilt spread, Stover[19] broadly divided the soils into resistant and non-resistant types. The disease spreads slowly in suppressive soils, but rapidly in conducive soils. One of the difference that distinguished these two types of soils was the calcium content. The calcium contents in suppressive soils was usually higher than those in conducive soils. It has been generally assumed without supporting evidences that calcium-deficiency in conducive soils is a contributing factor to wilt incidence. A beneficial effect of calcium on the fusarial wilt of tomato, caused by *F. oxysporum* f. sp. *lycopersici*, was shown by Jones and Woltz[9] who found that addition of lime to soil greatly decreased the incidence and severity of tomato wilt.

Because the ammonium sulfate has been extensively used as nitrogen source, the soils of banana plantations in southern Taiwan in general are lower in calcium and rather acidic. According to Stover (personal communication), the calcium level in Taiwan banana leaves was about 1/2 to 2/3 of that found in the Central Americas. The inadequate amount of calcium in soils and in plants may reduce the resistance of Taiwan Cavendishes to wilt. Field trial therefore was made to study the effect of liming on wilt incidence.

Five fields at different locations were chosen from the diseased areas. Lime (CaO) was applied at 5 tons per hectare and ploughed to the upper 30 cm of soil. The reaction of these soils before and

after liming was shown in Table 1. Planting of suckers was made at one month after liming. The results were negative as far as influencing disease incidence was concerned (Table 1). In one of these fields tested disease incidence was even higher in the limed than in the control plots. Replanting of banana was made in these field in the subsequent year and again the results were negative.

In a greenhouse assay involving the use of plantlets, Chuang (personal communication) also found that wilt incidence was not decreased with calcium content in soil increasing from 500 to 10,000 ppm. This is in agreement with those liming trials carried out in Central Americas where the Gros Michel was attacked by *F. oxysporum* f. sp. *cubense race* 1[15,18].

Table 1. The effect of soil liming on incidence of fusarial wilt of Cavendish banana

No. of plants tested	Wilt incidence (%)[1]	
	Limed plot	Control plot
155	12 (pH 7.1-7.7)[2]	11 (pH 5.7-6.3)
531	6 (pH 7.4-8.1)	3 (pH 7.0-7.8)
359	34 (pH 4.5-6.5)	44 (pH 4.2-5.5)
891	21 (pH 5.6-7.1)	24 (pH 4.3-6.4)
1050	45[3](pH 6.5-7.2)	26 (pH 5.5-6.6)

1) Data are averaged from two replicates in each field.
2) Soil pH was measured at shooting stage of banana plants, about 8 months after liming.
3) Significantly different from paired mean at 5% level by analysis of variance.

CONTROL BY CROP ROTATION

Fusarium oxysporum f. sp. *cubense* survives in soil for years. The longevity and level of population of the fungus is largely determined by such soil environmental factors as pH, soil type, and oxygen supply in soil, etc. Oxygen deficiency created by flooding or poor drainage resulted in a rapid decline of population[12]. This was once the practice in fusarial wilt control in Honduras, Panama, and other areas in South and Central Americas[19]. The banana plantations in southern Taiwan, once devastated by the wilt, were generally converted to paddy rice. The field soil was under flooded conditions for a period of about 5 months during two rice growing seasons in a year. The effectiveness of rice as a rotated crop for controlling fusarial wilt was investigated.

Replanting of bananas were made in 6 fields at different locations in the diseased area, each with 30-50% of wilt incidence upon abandonment of the banana crop. These fields had been in rice cropping continually for 1-3 years previously. Two sugar cane and one sunflower fields in adjacent area, that

also had been severely infected with fusarial wilt previously, were replanted with banana as non-flooded check. Suckers obtained from a wilt free orchard were used as planting materials. Results showed that percentages of wilt incidence in fields following rice were lower than that in fields following sugar cane or sunflower (Table 2-A). After one year in rice, wilt incidence was reduced to 8.1-17.6% from original 30-50%, and after 2.5 to 3 years it was further reduced to 0.8-6.3 %. No reduction in disease was evident in fields preceded by sugar cane and sunflower as rotation crops even for 3 years. Rishbeth[14] also reported that continuous planting of cane for 10 years did not control the disease.

Table 2-A. The effect of crop rotation on incidence of fusarial wilt of Taiwan Cavendish banana

Kind of crop and rotation period	No. of banana suckers planted[1]	Wilt incidence[2] (%)
Rice, 1 year	221	17.6
Rice, 1 year	132	9.8
Rice, 1 year	209	8.1
Rice, 1 year	229	15.3
Rice, 2.5 years	362	0.8
Rice, 3 years	320	6.3
Sugar cane, 3 years	435	38.6
Sugar cane, 3 years	616	70.9
Sunflower, 3 years	716	52.0

1) Suckers obtained from a wilt free orchard were used as planting materials.
2) Incidence of fusarial wilt of banana in these fields was 30-50% before rotating to other crops.

In another experiment, banana plantlets of 15-20 cm in height derived from meristem culture were planted to 6 diseased fields that had been in rice for 1.5-2 years previously. All these fields had a disease incidence > 20 % upon conversion to rice. Again, disease incidence was reduced to a very low level of 0-4.8% in most fields tested (Table 2-B). A 100% disease control was found in 2 fields that had been rotated with rice for 1.5 and 2 years, respectively. However, rotation of rice did not result in an effective disease control in a field located at Linpen, in which 20% of banana plants were wilted in the first year. This field was in immediate vicinity of other diseased banana fields during the testing period. Failure in disease control in this field may be attributed to the recontamination problem.

It is interesting to note that the disease usually appeared on or near the edge of former rice field. Tsai *et. al.*[25] also observed a same pattern of disease distribution in their control experiment with rice crop rotation. In preparation of

Table 2-B. The effect of crop rotation on incidence of fusarial wilt of Taiwan Cavendish banana

Location[1)]	Acreage[2)] (ha)	Rice cropping period (yr)	Wilt incidence (%)
TBRI exp. farm	0.2	2	0.3
TBRI exp. farm	0.1	2	0
Linlo	0.2	1.5	0
Wantan	0.25	2	4.8
Linpen	0.1	2	20
Chiatong	0.2	2	0.5

1) Field located at TBRI exp. farm were surrounded by other diseased banana fields, and were irrigated with underground water.
Fields located at Linlo, Wantan, and Chiatong were rather isolated, and were irrigated with surface water.
Field located at Linpen was surrounded by other diseased banana fields, and was irrigated with surface water.

2) Plantlets, 15-20 cm in height, raised from meristem culture were used as planting materials.

land for planting rice, incidentally it was found that most of diseased tissues were moved in water toward the edge when the field was plowed under flooded conditions. Consequently, the population of wilt pathogen in soil was higher on or near the edge than in the inner portion of the field. In the absence of host, the fungus was able to survive in non-flooded soil or in the roots of weeds commonly grown on the ridge[22]. This may explain why the infection first started from the edge in these fields.

SURVIVAL AND DISTRIBUTION OF THE PATHOGEN IN SOIL

With the replanting practice, abundant inoculum enters the soil from decaying diseased rhizome and pseudostem annually, and the inoculum potential in soil increases rapidly. As a result, the banana orchards in southern Taiwan, once infected, in general were destroyed in 3 years. By using the selective medium[23] for isolation from soil, the pathogen was found distributing primarily in the upper 20 cm of surface soil, and was not detectable in soil beneath this depth. Survival of the pathogen in soil is greatly influenced by the moisture. Hwang[5] reported that the fungus was able to survive for longer than one year in moderately moist soil, while it survived for 4 and 7 months only in submerged and dry soil, respectively. In a study of survival of the pathogen in the rice field, diseased soil containing 2.3×10^3 propagules/g soil, wrapped in nylon screen, were buried at various depths in soil. After one year in rice, 2% of the original fungal population remained surviving in the upper 3 cm of surface soil, while it was nondetectable in soil below 10 cm in depth. After 2 years in

rice, population of the fungus in surface soil was decreased further to nondetectable level. The yearly crop sequence in this field was rice-fallow-rice-bean during the testing period.

SELECTION FOR RESISTANCE

Appearance of the new race of *F. oxysporum* f. sp. *cubense* in Taiwan prompts the need to seek for resistant variety. The Taiwan Banana Research Institute has a collection of about 150 banana cultivars introduced from abroad. Hwang *et. al.*[6] showed that all the economic important cultivars belonging to the Cavendish group tested were susceptible to race 4 of the wilt pathogen, although some were highly resistant to race 1. Susceptibility to *F. oxysporum* f. sp. *cubense* race 4 of those introduced cultivars of economic importance, i. e. Dwarf Cavendish, Giant Cavendish, Grand Nain, Robusta, Valery, Veimama, Vietnam Chiao, and Williams Hybrid, were about the same as compared to that of Taiwan Cavendish[6]. The fact that none of existing economic cultivars tested possess any desirable level of wilt resistance suggests the need to seek resistance by other means. Due to the lack of resistant parent materials and the sterility inherited in triploids, breeding for resistance through conventional hybridization is extremely difficult and impractical in bananas. Efforts were made to seek source of wilt resistance from the somaclonal mutation occurring in nature or in meristem culture.

Selection of resistance from local commerical cultivars — Between 1974 and 1983, more than 3,000 selections were made from survivors in areas devastated by wilt in southern Taiwan. Suckers of wilt-free plant were obtained, and planted in a seriously diseased field. A sizable amount of diseased tissues was added to soil to maintain a high population of wilt pathogen in soil. Once wilt appeared at the first year the clone was discarded. From 1975 onward, only progeny showing more than 20% of disease out of 5-10 suckers tested was discarded. Clones showing 0 to 20% of disease were retained for further observation. By 1978 only 8 clones were left in the selection. In 1979, a total of 113 suckers obtained from these 8 clone were compared with 'check' plants in a grower's diseased field. Results showed that 90 out of 113 suckers were infected, and no clone remained wilt-free or had a lower percentage of wilt incidence. Several mutants of Taiwan Cavendish obtained from the local plantations were found also susceptible to the wilt[28].

Mass screen of plantlets from meristem culture — To prevent the spreading of fusarial wilt of banana by planting infected materials to the disease-free orchards, plantlets derived from meristem culture have been propagated for commercial planting in Taiwan since 1983[7]. A field survey showed that about 3% of banana plants grown from the plantlets were mutated[8]. Somaclonal mutation is an important potential source of disease resistance especially on such vegetatively propagated crops as bananas. With this rate of mutation generated from banana meristem culture, plantlets therefore are considered promising materials worth extensive screening for wilt resistance. That disease development in plantlets was faster than in suckers[24]

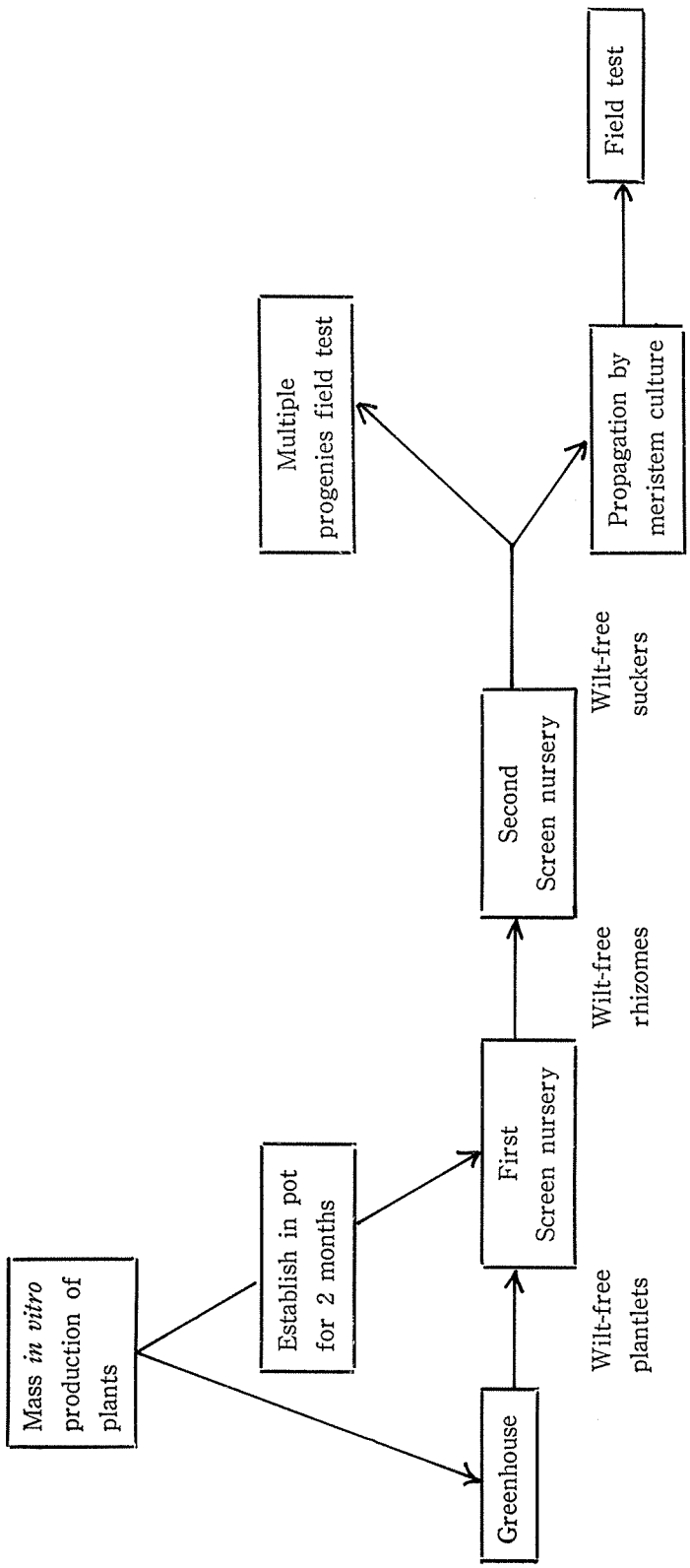


Fig 2. Screening of mass-produced *in vitro* plants of Cavendish varieties for resistance to race 4 of *Fusarium oxysporum* f. sp. *cubense*.

is also an advantage of using plantlets for screening purpose. Response of plantlets to different races of wilt pathogen was similar to that of suckers of the same variety[24].

The screen test was conducted under both field and greenhouse conditions (Fig. 2). Plantlets, propagated by the method as described by Hwang *et. al.*[7], were established initially in pot (9 cm high, 10 cm wide). The growing medium consisted of 60% vermiculite, 30% sand, and 10% organic manure by volume. Plantlets were maintained in the screenhouse for two months before transplanting to the field. The field soil had a content of fungal propagules at about 1200/g soil, ranging from 600-1500/g. Examination for vascular discoloration in the rhizome was made 4 months after planting. The plants with rhizome free from infection or with mild infection were replanted for further observation. In the greenhouse, plantlets taken out from the test tube were directly planted to the soil containing about 600 propagules/g soil, with a range of 400-1200 fungal propagules/g soil. Examination for disease was carried out after two months of growth. By using this method, a total of 13,878 plantlets of Taiwan Cavendish were screened in the first year, 17 of them remained free from infection. Further testing of these wilt-free clone is in progress.

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Ecology and Control of Gladiolus Fusarium Wilt

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摘　　要

謝式坪鈺（1985）唐菖蒲萎凋病之生態及防治　（國立中興大學）

由 *Fusarium oxysporum* f. sp. *gladioli* 引起之萎凋病爲本省唐菖蒲生產最大的難題。病菌經常汚染種植材料，可由病株或外觀健全植株之種皮、球莖和根部分離到病菌。不過病菌在病株球莖之含量常比外觀健全者高出 10 倍以上。

根由球莖長出後即可分離到病菌，病菌在土壤內主要分佈在表土至深 20公分處，到 30 公分深乃能分離到，球莖種植在不同深度的土壤影響其發芽與發病度。

溫水處理（57.2C）30 分鐘可消除球莖內之病菌，但處理後球莖之發芽率減低很多，對木子之影響較小。

單獨用福爾馬林或蒸氣土壤消毒並不能減輕病害之發生，添加 S-H 混合物並無法降低土壤內之病菌，也無法減少病害之發生。

農藥貝芬替、腐絕、鋅銅四氯丹、撲拉克和普拔克可有效制菌絲之生長，但只有撲拉克能抑制球莖組織內之病菌，利用撲拉克（有效成份 0.0125%）在種植前處理球莖 3 小時以上，可消除球莖內大多數病菌，田間試驗顯示對感病品種之防治率高達 50% 以上。

ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *gladioli* is a serious problem to gladiolus production in Taiwan. No control measure has been ever practical to this disease previously. The pathogen was usually associated with planting materials, and was commonly isolated from root, corm, husks of diseased and apparently healthy gladiolus. About 10 times more of the fungus was obtained from macerated diseased corms than from apparently healthy ones.

Colonization or infection of the fungus on roots was detectable immediately

after growing out from corms. The pathogen was present in soil mainly from surface to a depth of 20 cm and was detectable down to 30 cm deep. Corms planted at differrent depths in soil affected the germination of corms and incidence of disease.

Hot water treatment of planting materials at 57.2C for 30 min resulted in good control of the disease. However hot water treatment drastically reduced the germinability of the corms. Small cormels were more tolerant to hot water treatment than corms. Soil treatments with formalin and steam alone did not reduced the incidence of the disease. Soil amendment with S-H mixture at 0.5 and 1% neither reduced the population of the fungus in soil, nor decreased the incidence of the disease.

In the chemical screening tests, Bavistin, Mertect, Previcur N, Sportak and Zincofol were effective in inhibition of mycelial growth *in vitro*, however only Sportak was able to eradicate the pathogen from corm tissues. Corms treated with Sportak (0.0125% a. i.) for more than 3 hr before planting gave good control of the disease, the incidence being reduced up to 50% or higher on the susceptible varieties.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder & Hansen is an old and serious problem to gladiolus production in Taiwan. When susceptible varieties are grown, especially under continuous cultivation, the fungus may cause up to 50% losses or even completely destroy the plantation.

Growth of resistant varieties is the most effective way of controlling the disease. Unfortunately, most of the good quality varieties introduced from abroad are susceptible. Before the resistant varieties is available, adequate control other than seeking resistant varieties has to be developed.

The objectives of this study were to investigate the ecology of the causal organism; distribusion of F. *oxysporum* f. sp. *gladioli* in soil and its host plant; and to evaluate the effectiveness of various control methods for control the disease.

MATERIALS AND METHODS

PCNB[5] was used to isolate *F. oxysporum* f. sp. *gladioli* from plant materials and the soils. Identification of the pathogen was made by inoculating the isolates on corms or leaves of gladiolus. Span, a susceptible variety was used throughout the experiments except specifically indicated.

Qualitative and quantitative estimation of populations of the fungus in corms

Qualitative assay: The corms were vertically dissected from the center, and about 1 mm thick of the tissue was sectioned from the cut surface. This tissue section was further cut into 5 mm^2, and placed onto PCNB plates for incubation at 30C. The appearance of colonies of the pathogen on tissue sections was recorded 10 days later.

Quantitative assay: Sterile distilled water and corm tissues at ratio 20:1

(V/W) were homogenized in a high speed Waring Blender for 10 seconds and then subjected for serial dilution planting on PCNB plates. The colony counts were made 10 days after incubation at 30C.

RESULTS

Colonization and/or infection of gladiolus roots by *F. oxysporum* f. sp. *gladioli*

Fifty corms after broken their dormancy were placed on wet scott papers. After 1-, 3-, 5-, 7- and 9- day incubation 10 corms each were sampled, and their roots were cut and placed on PCNB plates to isolate the fungus. It was found that 34% of root was infected with the fungus 24 hr after germination of corms, and 50, 79, 71 and 75% of roots were infected 3, 5, 7 and 9 days after germination, respectively.

Density of *F. oxysporum* f. sp. *gladioli* in symptomless and diseased corms

Diseased plants and apparent healthy plants were sampled from field 2 months after planting. Individual corm was quantitatively assayed for density of *F. oxysporum* f. sp. *gladioli* on PCNB plates. The fungal population in diseased corms were $7.1\times10^4-4.2\times10^5$ propagules/g fresh weight. On the other hand, the fungal densities in apparent healthy corms were much less, $0-6.4\times10^3$ propagules/g fresh weight.

Distribution of *F. oxysporum* f. sp. *gladioli* in soil

Soils were sampled from Pei-tou, Tien-wei and Fou-li where gladiolus has been cultivated for many years. To analyze the vertical distribution of the fungus in soil. five soil samples were collected from different depth of the soil from surface to 30 cm depth with 5 cm intervals. These soil samples were subjected to serial dilution plating on PCNB plates. The fungus was mostly present in the upper 20 cm in depth; populations ranged from 2×10^2 to 2.6×10^3 propagules/g. At a depth of 20-30 cm, the population of propagules decreased to 0-300 propagules/g of soil. (Table 1)

Teble 1. Vertical distribution of *Fusarium oxysporum* f. sp. *gladioli* in soil

Depth of the soil (cm)	No. of propagules/g soil ($\times10^3$)		
	Pei-tou	Tien-wei	Fou-li
0-5	1.6	1.8	2.2
5-10	1.5	2.6	1.1
10-15	2.3	0.2	2.3
15-20	1.1	0.8	1.0
20-25	0.3	0.3	0.1
25-30	0.1	0.1	0

Planting of gladiolus corms in different depth of soil in relation to the occurrence of Fusarium wilt

Corms of Valeria and Hei-ku without any treatment, were planted in soil depth 4, 8, 12 and 16 cm, each consisting of 300 corms in 4 replicates at Pei-tou in 1983. The germination of corms was

recorded one month after planting, and percent of infection was estimated at flowering stage.

Of these two cultivars tested, the percentage of ungerminated corms and occurrence of the disease were increased with the increasing depth of planting in soil (Table 2). Valeria planted at 4 cm deep grew better than rest of treatments, and Hei-ku planted at depth from 4-12 cm grew better than that planted at 16 cm deep.

Table 2. Occurrence of gladiolus Fusarium wilt in different depth of soil

Variety	% of diseased plants or ungerminated corms in soil depth (cm)			
	4	8	12	16
Valeria no germination	22.5	26.3	23.8	31.3
diseased	5.0	15.0	20.0	13.8
subtotal	27.5a	41.3b	43.8b	45.1b[1)]
Hei-ku no germination	3.8	11.3	7.5	15.0
diseased	6.3	2.5	3.8	11.3
subtotal	10.1a	13.8a	11.3a	26.3b

1) Subtotals followed by a common letter do no differ significantly (P=0.05).

Effect of hot water treatment on the germination of corms and cormels and on the incidence of Fusarium wilt

Corms and cormels were kept at 35C for about one week immedately after harvest for curing. They were presoaked for 2 hr in water at room temperature and then in hot water at 57.2C for 30 min[1]. After thoroughly dried, they were cool storaged for 2 months. Half of the corms and cormels were qualitatively assayed for the presence of the fungus, and the rests were planted in the field for observation of the germinability and

Table 3. Effect of hot water treatment on the germination of corm and cormel and the incidence of Fusarium wilt

		% *F. oxysporum* f. sp. *gladioli* isolated	% germination	% disease
Cormel	treated	3	48	25
	CK	28	58	51
Corm[1)]	treated	78	16	4
	CK	100	53	42
Corm[2)]	treated	26	23	7
	CK	43	87	34

1) From diseased plants; 2) From apparent healthy healthy plants.
Data are means from two experiment of 100 cormels or corms each.

the disease incidence.

Hot water treatment drastically reduced the germinability of the corms, but has only slight influence on the cormels. When hot-water-treated planting materials were grown in the field, disease incidence was decreased. (Table 3)

Effect of SH mixture amendment on the population of *F. oxysporum* f. sp. *gladioli* in soil and the occurrence of Fusarium wilt

S-H mixture[6] was amended in soil at rate of 0, 0.5 and 1% (W/W) in field of Fou-li just before planting. Soil were sampled 2, 4 and 6 weeks after amendent and the serial dilution plating was made to estimate the population of the fungus, The percent of infection was recorded 2 months after planting. Amendment of soil with S-H mixture did not affect the population of the fungus in soil, neither did reduce the incidence of the disease.

Effect of soil treatments on germination of gladiolus corms and disease incidence

Soils previously grown gladiolus for 3 years at Pu-li were disinfested with steam or formalin. After disinfestation, isolation was made on PCNB to ensure that there was no any *Fusarium* detectable in treated soil. The control was provided by using the same field soil without disinfestation, containing about 5×10^2 propagules/g of *F. oxysporum* f. sp. *gladioli*. The experimental design was completely randomized with four replicates, with 200 corms per replicate. Soil sterilization with formalin and steam to eliminate the pathogen did not affect the germination of corms and the incidence of the disease. There was no significant difference in percentage of germination and percentage of disease among formalin treatment, steam treatment and the control (Table 4).

Table 4. Germination of gladiolus in formalin and steam disinfected soils and incidence of Fusarium wilt

Treatment	% germination	% diseased
Formalin	83 a	53 a[1)]
Steam	85 a	60 a
CK	80 a	58 a

1) Based on 200 corms in each treatment. Colomn means followed by a common letter do not differ significantly (P=0.05).

Evaluation of fungicides for controlling Fusarium wilt of gladiolus

Paper discs in 5 mm diameter were soaked in 0, 0.1 or 0.067% suspension of Benlate (50% Benomyl), Bavistin (50% Carbendazim), Mertect (40% Thiabendazole), Difolatan (40% Captafol), Captan (50% Captan), Zincofol (50% Captafol+6% Zinc+12.% Copper), Previcur N. (70% Propamocarb) and Sportak (25% Prochloraz), for 1 hr., then removed and placed on a nylon screen for drying. After dryness, they were placed on Petri dish containing 10 ml of PSA to which approximately 100 macroconidia of the fungus were added. The

inhibition zone was measured from edge of paper discs 10 days after incubation at 28 C. Sportak was the most effective followed by Mertect, Previcur N, Bavistin and Zincofol (Table 5). In another experiment, corms were soaked in fungicides for 3 hr. and then qualitatively assayed for the presence of the fungus in corm tissues. Sportak was the most effective in eradicating the pathogen in corm tissues. Sportak at 125 ppm eliminated about 70% of pathogen in corm tissues, whereas other fungicices did not significantly reduce the percentage of isolation, as compared with the check (Table 6).

Table 5. Effect of fungicides on the inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *gladioli*

Fungicide	Inhibition zone (mm) at concentration		
	500X	1000X	1500X
Bavistin	14.4	14.0	12.8
Benlate	5.6	1.0	1.6
Captan	6.8	6.8	6.8
Difolatan	9.8	9.0	8.0
Mertect	18.2	13.0	14.4
Previcur N	17.3	15.6	10.2
Sportak	18.5	18.4	17.7
Zincofol	13.0	10.5	12.0

Table 6. Frequeny of isolation of *Fusarium oxysporum* f. sp. *gladioli* from corms treated with various fungicides for 3 hr

Fungicides	Concentration (a. i.)	% isolation[1)]
Bavistin	500 ppm	91
Mertect	400 ppm	87
Previcur N	800 ppm	86
Sportak	125 ppm	30
Zincofol	500 ppm	94
CK		99

1) Based on 100 isolations.

Isolation of the fungus from old corms or newly formed corms and cormels

To understand the effect of fungicidal treatment on the subsequent crop, corms after Sportak treatment for 3 or 24 hrs were planted in the field. After about 4 months, old corms as well as newly formed corms were qualitatively assayed for the presence of the fungus. The

fungus was present in 95% old corms and 75% newly formed corms in the control block-without fungicidal treatment. Whereas, in those treated with Sportak for 24 hr, it was isolated from 24% old corms and from 12% newly formed corms (Table 7). Sportak treatment for 24 hr gave better control than treatment for 3 hr only.

Table 7. Isolation of *Fusarium oxysporum* f. sp. *gladioli* from old and newly formed corms produced from plants previously treated with Sportak before planting

Duration of treatment before planting (hr)	% isolated from	
	Old corms	Newly formed corms
0	95	75
3	83	54
24	24	12

Duration of Sportak treatment and the occurrence of Fusarium wilt

Corms before planting were soaked in Sportak (0.0125% a. i.) for 3, 6, 12 and 24 hrs. Corms soaked in tape water for 24 hr, or without soaking served as control. The experimental design was completely randomized with five replicates. Germination of corms was observed one month after planting, and percentage of disease was recorded at flowering stage. The results showed that 3 hr treatment was sufficient to control the disease. and longer treatment did not further reduce the incidence of the diseases. Prolongation of treatment reduced the germination of corms (Table 8).

Table 8. Duration of treatment of corms in Sportak in relation to the gladiolus Fusarium wilt

Duration time (hr)	% Ungerminated	% Diseased	% Ungerminated + Diseased
3	15.0 a	2.5 a[3)]	17.5
6	13.0 a	3.0 a	16.0
12	14.5 a	3.5 a	18.0
24	24.5 b	1.5 a	26.0
0[1)]	22.0 b	48.5 b	70.5
0[2)]	29.5 b	54.5 b	84.0

1) Without soaking.
2) Soaking in water for 24 hr.
3) Column means followed by a common letter do not differ significantly (P=0.05).

Field trial of Sportak treatment on the control of the disease

Two field trials were conducted at Pei-tou and Fou-li in 1983. The experimental design was completely randomized with four replicates. Each replicate had 150 plants. Corms were soaked in Sportak (0.0125% a. i.) for 3 hr before planting. Corms soaked in irrigation water for 3 hr were used as control. The percentage of disease and number of harvestable cut flowers were recorded at flowering stage. The results showed that Sportak gave a satisfactory control of the disease, with only 17.4% plants infected with the fungus as compared with 68.7% infection in control (Table 9). The average number of cut flowers in treated block was 130, and it was 28 only in control block.

Table 9. Field trial of the effectiveness Sportak treatment on the control of gladiolus Fusarium wilt

Treatment	% Diseased	No. cut flowers
Sportak	17.4 a	130 a[1)]
CK	68.7 b	28 b

1) Data are means from two experiments of four replicates. Each replicate has 150 plants. Column means followed by a common letter do not differ significantly (P=0.05).

DISCUSSION

Fusarium oxysporum f. sp. *gladioli* was isolated from root, corm tissues, husks of diseased and apparently healthy gladiolus. The pathogen was carried over through the planting materials, a typical corm-borne disease. More than 50% of roots were infected by the pathogen immediately after growing out from the corms. The pathogen may enter into soil from infected husks and root tissues. This explains why the pathogen was not present in virgin soil but was detectable immediatdly after planting of gladiolus. (Unpublished data)

From the unpublished data, it was shown that the pathogen was also isolated from corm tissue of resistant varieties. This indicates that the corms of resistant varieties might be also infected by the pathogen, but remained symptomless. The symptoms would not appear at the early stage of infection when the density of fungus in the tissue was not high. The fungual population in corm from diseased plants was 10 times more than that from apparent healthy ones. The pathogen in corm tissues has to multiply up to certain levels before showing symptoms. The pathogen population in diseased corms is always more than 10^4 propagules/g fresh tissues. The bacterial population in diseased corm was also much higher than that in apparently healthy corm. The bacteria from corm tissue were mostly non-pathogenic, but occasionally *Xanthomonas gummisudans* was observed. The role of the bacteria in diseased corm tissue has to be identified.

The population of *F. oxysporum* f. sp. *gladioli* in soil was mostly distributed from surface to a depth of 20 cm, in concomitance with the distribution of root system of the gladiolus. Planting corms in shallower soil seemed to be better than those in deeper soil. However from practical point of view gladiolus has to be planted at deeper soil as plants may fall when planted at shallow soil.

Hot water treatment at 57.2C for 30 min effectly control the disease, however it reduced the germinability of the corms. These results were similar to that of Magie[3]. However, hot water treatment is not practical as the equipment is usually not available and it is time consuming.

Amendment of soil with S-H mixture at 0.5 and 1% did not reduce the population of the fungus in soil and the incidence of the disease. This was different from the results of Sun & Huang [6] who reported that S-H mixture amendment reduced *Fusarium oxysporum* f. sp. *niveum* population in soils.

Planting untreated corms in formalin and steam sterilized soils did not reduce the incidence of the disease. Similar results were obtained by Milholland[4], Ferguson and Markley[2] who showed that planting untreated corms in fumigated soil resulted in an increase rather than a reduction of disease. Therefore soil fumigation should not be used when untreated corms are to be planted. The best results can be expected through planting clean corms in sterilized soils.

Among various fungicides tested, Sportak not only effectively inhibited the mycelial growth *in vitro* but also eliminated most of the fungus in corm tissues. The corms treated with Sportak (0.0125 % a. i.) for 3 hr before planting was sufficient to control the disease, the incidence being reduced up to more than 50% on the susceptible varieties. Prolongation of treatment did not further increase the effectiveness of the disease control, although it reduced the fungal population in treated corms and newly formed corms of subsequent crop. Since Sportak is not a systemic fungicide, presene of Sportak in corm tissue mainly relies on passive diffussion. The treatment of Sportak would be better conducted when corm has partially lost its water content and has a greater water absorption capacity. When treated with Sportak immediately after harvest, corms were found to have more fungus uneradicated than those after storage. Sportak was unable to eradicate the fungus in corms completely; it is recommended that the corms be treated before planting at each crop.

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Ecology and Control of *Phytophthora melonis* in Drained Paddy Field[1)]

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摘　　要

林益昇、吳瑞香 (1985) ***Phytophthora melonis*** 在水田裡之生態及防治。（臺灣省農業試驗所）

胡瓜疫病菌 (*Phytophthora melonis*) 之游走子囊在病組織上不具脫落性，乾風無法將之吹落。但是它的游走子及游走子囊可隨灌溉水及飛濺的雨水傳播。土壤濕度強烈影響病原菌的存活，病原菌在淹水的土壤中僅能存活 4 星期，但在含水量 6 %及 20%，稍可通氣之濕土裡，也僅能存活 5～20 星期。田間試驗顯示，在經過兩期水稻輪作的病圃裡，胡瓜疫病菌不能存活。而引起胡瓜疫病之病原菌初次感染源，並非來自土壤或種子，而是來自隣田的灌溉水。在田間，殺菌劑(Ridomil MZ)和土壤添加物(雞糞和 S-H 混合物) 配合使用對胡瓜疫病之防治效果最顯着。該二種土壤添加物可提升土壤的 pH 值，但土壤 pH 值的變化與病害防治無關。雞糞和 S-H 混合物僅有促進植株生長勢的效果。在試驗中 Ridomil MZ 殺菌劑才是抑病的主要因素。

（關鍵字：胡瓜疫病，*Phytophthora melonis*，初次感染源，存活，病害傳播，病害防治，水稻田）

ABSTRACT

Dry air current failed to remove the sporangia of *Phytophthora melonis* from the disease lesions. However, the fungus was easily dispersed by its zoospores and sporangia carried in irrigation water or liberated to the air by

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wind-blown rainsplashes. The soil moisture strongly influenced the survival of *P. melonis*. The fungus persisted only for 4 wks in flooded soil, while in soil with moisture content at 6% or 20%, a condition that permitted soil aeration, the fungus could be detected from the infested soil for a period of 5~20 wks. Field experiments demonstrated that *P. melonis* did not survive, nor did it induce cucumber blight in a disease nursery after two successive crops of rice plants. The primary inoculum of *P. melonis* to cause cucumber blight in the drained paddy fields did not come from the paddy soil nor from cucumber seeds. It was mainly from irrigation water from neighbor fields. Cucumber blight was best controlled by using combinations of fungicide Ridomil MZ and soil amendments, i. e. chicken manure or S-H mixture. The soil amendments raised soil pH to alkaline range for 7 days, then it fell to the acid range again. The changing pattern of soil pH had no correspondence to the inhibition of cucumber blight. Both chicken manure and S-H mixture had nutrient effects to result in improved plant growth. However, Ridomil MZ was the main factor that inhibited disease development in our experiments.

(Key words: Phytophthora blight of cucumber, *Phytophthora melonis*, primary inoculum, survival, spread of disease, disease control, paddy field)

INTRODUCTION

Cucumber is an important vegetable in Taiwan with an annual acreage of about 5,000 ha[9]. It is mostly grown in the drained paddy fields. The population of many soilborne pathogens in soil can be considerably reduced following the cropping of paddy rice[3,11]. However, Phytophthora blight of cucumber caused by *Phytophthora melonis* Katsura was common on cucumber grown under the above conditions. The blight symptoms were found on all aerial parts of the plant. Severe occurrence of the disease was observed mostly during periods of hot wet conditions[6]. In our preliminary study, *P. melonis* could not be reisolated 4 wks after flooding the field, when the organism was grown on cucumber fruits and burried in paddy soil. How does the fungus survive in paddy soil is of great interest. In addition, the fungicide Terrazole, recommended for controlling the disease[7], was found not satisfactory in field trials. We therefore, investigated the form of primary inoculum, the survival and dispersal of *P. melonis*; the way of disease spread; and the control measures which could be adopted under conditions of growing cucumber in the paddy field between rice crops.

MATERIALS AND METHODS

Inoculation of detached cucumber leaves

Zoospores of *P. melonis* (homothallic isolate Phy-83-13) were prepared by the method of Hwang and Ko[4] into a dilution series. A drop (0.035 ml) of the diluted spore suspension containing either 1-10 or 48-56 zoospores was taken up with a microsyringe and placed onto the abaxial or adaxial surface of the detached

cucumber leaves. The inoculated leaves were kept in a moist chamber at 28 C, and symptoms were recorded 4 days after inoculation.

Dispersal of sporangia and zoospores

In the laboratory, an inoculated cucumber fruit covering with sporangia of *P. melonis* was hand-held in an upright position 10 cm from a petri dish, then a fan blew the air at various speeds over the fruit in the direction of the petri dish. In another experiment, inoculated cucumber fruits were placed on a holder 10 cm above a tray which was put in the open under heavy rain for an hour. The rain-splashes were collected in the tray. The water collected in both the petri dish and the tray was examined microscopically for the presence of sporangia and zoospores.

Spread of Phytophthora cucumber blight

Cucumber seeds (cultivar Joy) were sown in the greenhouse (28~38 C) in 3 rows (3 m long for each, 30 cm between rows). The plants of the middle row were cut off 21 days after seeding and one inoculated cucumber fruit was placed between the first and second rows at the entrance of irrigation water. No inoculum was placed between the second and third rows. The plot was irrigated carefully every other days. Water samples were collected at the end of irrigation path and passed through a 400-mesh screen. The materials collected on the screen was examined under microscope for sporangia. Attempt was also made to isolate the fungus from the water samples by baiting with cucumber leaves. The disease development was recorded during the experimental period.

Cucumber fruits were inoculated and placed at the irrigation entrance of a cucumber field (0.2 ha) during heavy raining days. Water samples were also collected from the furrows between rows and examined for the presence of the fungus zoospores and sporangia as described. The disease development in the field was recorded. When the cucumber plants were 2-month old and the disease started to develop, a home-made simple spore trap equipped with 8 petri dishes (9 cm in diam.) was installed 30-cm, 40-cm, 60-cm and 80-cm (two petri dishes at each height) above the ground in the center of the nursery. The airborne inocula were collected on fresh Phytophthora selective medium[5] in the petri dishes during a 12 hr exposure. The petri dishes were then examined under microscope for the presence of sporangia and zoospores. The collections were repeated 10 times in both sunny and raining days.

Survival of *P. melonis*

(a) Ten samples with a total of 4,500 cucumber seeds obtained from local seed stores were put on selective medium[5] for isolating *P. melonis*.

(b) Cucumber fruits were inoculated with *P. melonis* in the laboratory and, after 5 days, chopped into small pieces (3 cm long). Then, the pieces of inoculum were subjected to the following treatments: (i) being immersed in tap water; (ii) placed on soil surface; and (iii) burried under soil at 2 cm depth. The soil moisture content was maintained at 10%, 20% or 30% (flooded). All infested materials were separately put in a one-liter beaker and incubated at room temprature (24~28 C). The beaker was

sealed with a plastic sheet to prevent the loss of moisture. The inoculated tissues were examined under microscope once a week for the presence of sporangia and zoospores.

(c) Autoclaved cucumber stems (2 cm long) were inoculated with *P. melonis* and mixed in a one-liter beaker with soil. The mixture was incubated at the laboratory temperature of 18~32 C (August, 1984 to Janurary, 1985). The soil moisture content was kept at 6%, 20% or 30%. Attempts were made to reisolate the pathogen from the infested soil at weekly intervals by baiting with cucumber leaves.

(d) The survival and primary inoculum of *P. melonis* were also investigated under paddy conditions. Two successive crops of rice were grown in the blight nursery (0.2 ha) in 1984. This same field was grown with cucumber plants during March to June 1985. Occurrence of cucumber blight was recorded and attempts were made to isolate *P. melonis* during the growing period of the first season rice and cucumber from the field soil as well as from irrigation water before entering into the field.

Experiments on disease control

Field experiments were conducted on an experimental farm (0.2 ha) of Taiwan Agricultural Research Institute during May to July and September to November of 1983, and May to July of 1984. Eggplant fruits were inoculated with *P. melonis* (homothallic isolate Phy-83-13) and incubated in the laboratory (24~28 C) for 2 days. The inoculated fruits then were blended with noncropped loam soil (1 fruit/100 g soil) to serve as soil-inoculum. A disease nursery was set up by adding fresh soil inoculum into seeding rows (0.4 kg/m) at 5 cm depth from the soil surface. Three experiments were set up in this disease nursery to evaluate the effect on cucumber blight for chicken manure (2,000 Kg/ha), cow dung (3,500 Kg/ha) and S-H mixture[8] (900 Kg/ha) as soil amendments; two fungicides, Ridomil MZ (Methyl D, L-N-(2,6-dimethyl-phenyl)-N-(2'-methoxyacetyl)-alaninate, 10%; Ionic coordination of zinc and manganese ethylenebisdithiocarbamate, 48%)(1450 ppm) and Terrazole (5-Ethoxy-3-(trichloromethyl)-1, 2, 4-thiadiazole, 35%)(175 ppm), and combined applications of fungicides and soil amendments. In the first experiment (12 m × 50 m), the three soil amendments were separately added to the soil between seeding rows (20 cm away from the rows one day before seeding. The second experiment (8 m × 50 m) used no soil amendments, but each seeding hole was drenched with 100 ml of one of the test fungicides right after seeding. The same fungicide was applied as leaf sprays to cucumber plants 3 times at a 10-day interval. In the third experiment (20 m × 50 m), all possible combinations of soil amendments and fungicides were included. One plot which received neither soil amendments nor fungicides served as check in all experiments. Each treatment (plot) employed 100 plants in 2 rows. Each experiment was a complete random design with 5 replicates. The disease intensity was calculated by the following formula:

$$\text{Disease intensity} = \frac{0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3}{(n_0 + n_1 + n_2 + n_3) \times 4} \times 100\%$$

Where: n_0 = No. of healthy plants.

n_1=No. of plants with water-soaking lesions.

n_2=No. of plants with shriveled stems.

n_3=No. of wilted plants.

In the greenhouse (25~38 C), a noncropped loam soil was amended with chicken manure (1/4, v/v) or S-H mixture (1%, w/w) before potting. Fresh soil inoculum was then added to potted soil at a depth of 5 cm. To each 6-in clay pot, 5 cucumber seeds were sown. After seeding, each pot received 100 ml of Ridomil MZ (1450 ppm) or Terrazole (175 ppm). Disease development was recorded and the soil pH was measured during the experimental period.

RESULTS

Infection of detached cucumber leaves

The inoculation results showed that successful infection with *P. melonis* could be induced in detached cucumber leaves by as little as 1-10 zoospores in the inoculum, though a higher disease incidence resulted from an inoculum containing a higher number of zoospores.

Dispersal of sporangia and zoospores

Airborne sporangia were not detected in both the laboratory and the field when recovery experiments were carried out in windy, sunny days. By contrast, airborne sporangia and zoospores were easily obtained from rain-splashes reaching the tray and the spore trap in raining days. The collected airborne sporangia did not have 'pedicel' or the 'pedicel' was not uniform in length.

Spread of Phytophthora cucumber blight

In the greenhouse, all plants in the first row which was near the initial inoculum showed root rot symptoms and were killed in 2-3 wks, while those plants in the third row remained healthy. In the waste irrigation water, the fungus could be detected by the baiting tecnique, but no sporangium was observed under microscope. In the field, similar results were obtained. Foot rot occurred first in the plants near the inoculum, then it spread to other plants of the same row following the path of irrigation water. After several days of heavy rains, however, the disease spread all over the plots, and the diseased plants developed foot rot as well as blight symptoms on the aerial parts. In the water samples collected from the furrows between rows, the sporangia and zoospores of *P. melonis* were observed under microscope. They were also obtained on culture medium.

Survival of *P. melonis*

(a) In tests for seedborne nature of *P. melonis*, all 4,500 cucumber seeds plated were found free from the pathogen.

(b) Cucumber fruits inoculated with *P. melonis* produced abundant sporangia in water or moist soil in 7 days. The number of sporangia was reduced to a very low level by 14 days (Table 1), especially in flooded soil. No oospore was formed at 7 days on the inoculated fruits irrespective of the latter being burried in soil with a 10% or 20% moisture content or in soil flooded with water. However, numerous oospores were produced during 7-14 days under the first two moisture conditions. There was no oospore found in the flooded soil (Table 1). After 14 days, the infected cucumber fruits had decayed in the soil,

Table 1. Effect of soil moisture on the formation of sporangia and oospores of *Phytophthora melonis*[1)]

Duration (day)	Soil moisture (%)	Treatment[2)]					
		In water		On soil surface		Buried in soil	
		S.	O.	S.	O.	S.	O.
7	10			+++	−	+++	−
	20			+++	−	+++	−
	30 (flooded)			++	−	++	−
	CK (no soil)	+++	−				
14	10			+	++	+	+
	20			+	+	+	−
	30 (flooded)			−	−	−	−
	CK (no soil)	+	−				

1) The experiment was conducted in laboratory (25~28 C) using homothallic isolate Phy-83-13.

2) *Phytophthora melonis* was grown in cucumber fruits for 5 days, then the fruits were put in water (CK), on soil surface or in soil. +++, ++, + and − indicate more than 50, 10-50, 1-10 and 0 sporangia or oospores per microscopic field (100X), respectively. S=Sporangia; O=Oospores.

the examination of the fungal structures was difficult.

(c) *Phytophthora melonis* was detected from the soil up to 20, 5, and 4 wks after infestation with inoculated cucumber stems for soil moistures at 6%, 20% and 30%, respectively (Table 2). The fungus was not recovered at later times.

Table 2. *Phytophthora melonis* reisolated from infested soil of different moisture content[1)]

Duration (wk)	Soil moisture (%)		
	6	20	30
3	+	+	+
4	+	+	+
5	+	+	−
6	+	−	−
7	+	−	−
8	+	−	−
10	+	−	−
20	+	−	−
22	−	−	−

1) *Phytophthora melonis* was grown on autoclaved cucumber stems for 5 days, then mixed with soil in laboratory. The pathogen was reisolated by baiting with cucumber leaves. +, fungus obtained; −, fungus not obtained.

(d) Three weeks after flooding during the first season of rice crop, *Phytophthora melonis* could not be detected in the paddy soil, previouly established as disease nursery. However the organism was detected in soil samples taken from the border of the paddy at least for 6 wks. This field was sown with cucumber seeds on March 24, 1985 and no *P. melonis* was detected from the irrigation water or soil samples at the seeding date. The plants grew well for 2 mo after seeding. Then, on May 21, *P. melonis* was first isolated from irrigation water at its entrance into the experimental field and cucumber blight occurred on May 28. All plants in this field were killed by June 15.

Experiments on disease control

Field experiments showed that none of the soil amendments or fungicides, when applied alone, was effective in controlling cucumber blight, except that Ridomil MZ treatment slowed down the disease development to some extent (Fig. 1A, 1B). Ridomil MZ plus soil amendments, i. e., chicken manure, cow dung and S-H mixture, showed better disease control (Fig. 1C), while Ridomil MZ plus chicken manure yielded best (Fig. 2).

Greenhouse experiments gave similar results. Ridomil MZ was of value in controlling cucumber blight. Soil amended with chicken manure and S-H mixture raised the soil pH from the original 6.1 to 7.5 and 7.8 for 1 wk, respectively, then it fell to 6.3 and 6.5, respectively. The various soil amendments had a little or no economic value in controlling the disease.

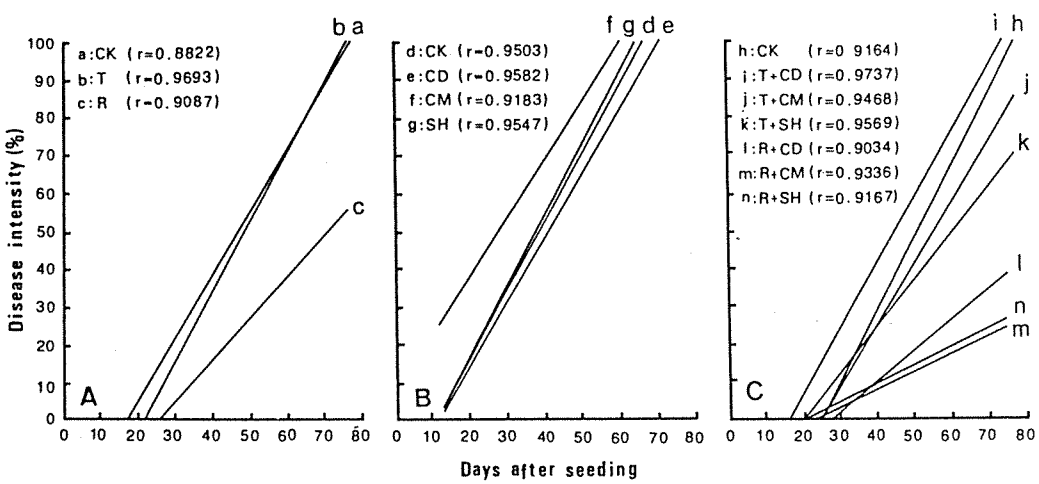

Fig. 1. Development of Phytophthora blight of cucumber in a disease nursery treated with fungicides (A) [Ridomil MZ (R) and Terrazole (T)], soil amendments (B) [chicken manure (CM), cow dung (CD) and S-H mixture (SH)], or combinations of soil amendment and fungicide (C) during March to June of 1983 in an experimental farm of Taiwan Agricultural Research Institute.

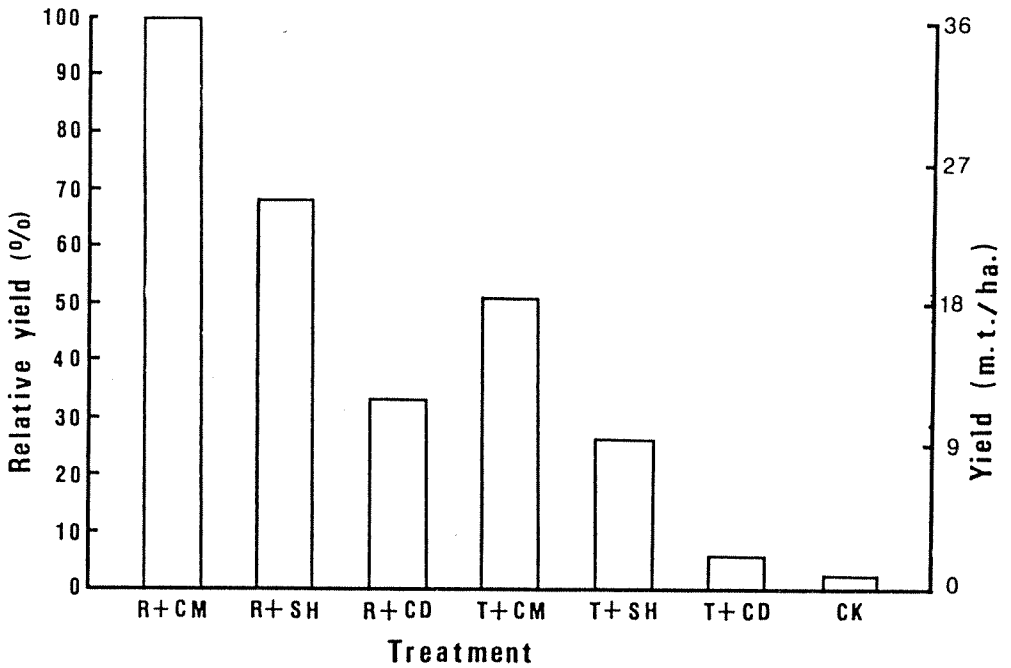

Fig. 2. Yield of cucumber in a disease nursery treated with combinations of soil amendment [chicken manure (CM), cow dung (CD) and S-H mixture (SH)] and fungicide [Ridomil MZ (R) and Terrazole (T)] during March to June of 1983 in an experimental farm of Taiwan Agricultural Research Institute.

DISCUSSION

That the sporangia of *P. melonis* could not be trapped by exposing agar plates at various heights above the ground in a disease nursery in sunny days, nor was the attempted collection of them successful in the laboratory by blowing dry air over the infected, sporangia-bearing cucumber fruits toward petri dishes provides evidence for the non-caducous nature of this fungus structure. However, they could be found in rain water passing over the surface of an infected cucumber fruit and collected in the tray situated below. The sporangia thus collected had no 'pedicel' or their 'pedicel' was not uniform in length, suggesting that they were probably broken by rain drops impeaching the disease lesions[1]. Other *Phytophthora* species[2] were also found not to release the sporangia into air current under dry condition, but they were readily dispersed by wind-blown rain-splashes. In our field experiments, *P. melonis* was found to be dispersed by zoospores and sporangia carried in irrigation water, although only the zoospores, not the sporangia, were found in greenhouse experiments. In Taiwan, rains and showers occur frequently in the spring and summer seasons, the zoospores and sporangia either carried in irrigation water or liberated to the air by wind-blown rain drops become an important means of

short distance dispersal in the field.

The soil moisture strongly influenced the survival of *P. melonis*. When the inoculum was put in water or in flooded soil, *P. melonis* would undergo vegetative growth leading to the production of sporangia and soon lysed (Table 1). By contrast, when the inoculum was burried in moist soil (10% and 20% of moisture content), the fungus would produce sporangia within 7 days, followed by the formation of many oospores during 7~14 days (Table 1). However, it is difficult to determine the survival time period of oospores of *P. melonis* in soil since their germination rate was extremely low, only 0.1% on cucumber extract agar. In our study, survival of the fungus was determined by first infesting the soil with the fungus grown in autoclaved cucumber stems and reisolation made at times by the baiting technique. In this way, the fungus could be detected over a period of 20 wks (Table 2) from the infested soil at 6% moisture content. Under the field conditions, *P. melonis* did not survive, nor induce cucumber blight in a disease nursery after two succcessive crops of rice plants. The primary inoculum to cause infection later in the experiment on cucumber plants as a succeeding crop is believed not from the soil or the cucumber seeds. It was mainly from the irrigation water which came from neighbor fields.

It has been shown previously that amendment of soil with chicken manure [10] or S-H mixture[8] raised the soil pH to alkaline range temporarily. A relatively high soil pH may either eliminate or significantly reduce *Phytophthora*[10] and *Fusarium*[8] propagules in the soil. Our greenhouse and field experiments showed that addition of chicken manure or S-H mixture raised the soil pH for a period of about 7 days, it then fell to the acid range. But this changing pattern of soil pH had no correspondance to the inhibition of cucumber blight caused by *P. melonis*. The reason may be that the fungus is not soilborne in nature and hence the soil amendments have little effect on the fungus coming from a neighbor field. However, both chicken manure and S-H mixture improved plant growth. Ridomil MZ was the main factor to inhibit the disease in our experiments.

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Bacteriocin-producing Strains of *Pseudomonas solanacearum* and Their Effect on Development of Bacterial Wilt of Tomato

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摘　　要

蔡正文、徐世典、陳隆鐘（1985）。**產生細菌素之青枯病菌菌株及其對番茄青枯病發生之影響**。（國立中興大學植物病理學系）

以臺灣各地區六種寄主植物上分離的青枯病菌 52個菌株作爲指示菌株時，所有菌株均能產生細菌素，但產生的細菌素其抑制作用範圍差異甚大，其中以天堂鳥花分離的六個菌株所產生的細菌素最具廣效性，它們能抑制52個菌株中 73～90%菌株的生長。從這六個天堂鳥花菌株再分別選取而得的無致病性菌株仍具有產生細菌素之能力（Avirulent bacteriocin-producing strains, ABPS)。當番茄幼苗根部浸漬於 ABPS 懸浮液（10^9 cfu/*ml*）中 30分鐘後再種植於含有青枯病菌的土壤中時，都能延遲發病或減輕發病程度，但其防治效果因不同的 ABPS 而有差異，其中以 BP5A 的防治效果最佳。如以另一無致病性但不產生細菌素的青枯病菌菌株 PS31A 作同樣防治試驗，則無防治效果。BP5A 雖有良好的防治效果，但其效果不能持續到植株生長的後期。BP5A 接種於番茄根部後，其族群逐漸降低。

（關鍵字：青枯病菌，細菌素，番茄，生物防治）

ABSTRACT

Fifty-two strains of *Pseudomonas solanacearum* collected from six hosts in different parts of Taiwan were assayed for bacteriocin activity using all of the 52 strains as indicators. Each strain was found to produce bacteriocin against at least one of the 52 indicator strains. The test strains varied considerably in their spectra of bacteriocin activity, among them, 6 strains isolated from bird-of-paradise inhibited the growth of a greatest number of *P. solanacearum* strains. These 6 strains were selected for further study and an avirulent strain was obtained from each of the 6 strains. The avirulent strains retained their wide spectra of bacteriocin activity when tested against all of

the 52 strains. Effect of the 6 avirulent bacteriocin-producing strains (ABPS) on development of bacterial wilt of tomato was evaluated in the greenhouse. Disease development was delayed or disease severity was reduced when roots of tomato seedlings were dipped in suspensions of ABPS (10^9 cfu/ml) prior to transplanting in soil infested with virulent strain PS68 (a bacteriocin-sensitive strain) of *P. solanacearum*. The level of disease control, however, varied with the ABPS, of which, BP5A showed the best control. When an avirulent bacteriocin-nonproducing strain (PS31A) selected from virulent strain PS31 was tested for control of the disease by the same root dip method, PS31A was not able to suppress the disease. Although BP5A was effective in protecting tomato seedlings from bacterial wilt, the protection did not persist to the later stages of tomato growth. The population of BP5A on the roots declined gradually after treatment.

(Key words: *Pseudomonas solanacearum*, bacteriocin production, tomato, biocontrol)

INTRODUCTION

Bacterial wilt of tomato caused by *Pseudomonas solanacearum* (Smith) Smith is one of the most important bacterial diseases in Taiwan. The disease has often been the limiting factor in tomato production in summer or warm seasons. The disease is also of major economic importance on several other solanaceous crops[6]. Breeding for disease resistance is the main approach for controlling bacterial wilt in Taiwan. During the past 10 years good progress has been made in breeding tomato for resistance to the disease[11,12,13]. However, highly resistant cultivars have not yet been developed. While breeding for resistance is the most important method for bacterial wilt control, other means of control may be necessary and used with host resistance in an integrated approach to disease management.

The potential use of avirulent bacteriocin-producing bacteria for control of bacterial plant diseases has been suggested[16]. The successful control of crown gall disease of various crops incited by *Agrobacterium tumefaciens* (Smith & Townsend) Conn by preplanting treatment with a nonpathogenic bacteriocin-producing strain 84 of *A. radiobacter* (Beijerinck & van Delden) Conn is a well-known example[10,15]. Recently, Chen et al[1] reported that tobacco seedlings were protected from bacterial wilt by immersion of roots of the seedlings into cell suspensions of avirulent bacteriocin-producing strain of *P. solanacearum* before planting. The purposes of this study were to determine whether strains of *P. solanacearum* collected in Taiwan are bacteriocinogenic and to evaluate the avirulent bacteriocin-producing strains of this bacterium for control of bacterial wilt of tomato.

MATERIALS AND METHODS

Bacterial strains Strains of *P. solanacearum* were obtained from the stock culture collection maintained in the Department of Plant Pathology at the

National Chung Hsing University. They were isolated from various host plants in different parts of Taiwan and maintained in sterile distilled water in screw cap tubes at room temperature. The sources of the 52 strains tested for bacteriocin production and sensitivity were as follows: PS9, 11, 21, 27, 31, 38, 41, 50A, 50B, 64, 65, 66, 67, 68, 69, 73, 79 and 82 (from tomato collected in Taichung area); PS44, 45, 48 and 51 (tobacco, Taichung); PS60 and 98 (potato, Nantou); PS61 (tomato, Nantou); PS63 (sweet pepper, Nantou); PS70 (tomato, Changhua); PS71 and 72 (tomato, Yunlin); PS75 (tomato, Tainan); PS76 and 77 (eggplant, Pingtung); PS78 (tomato, Pingtung); PS84 and 86 (sweet pepper, Taichung); PS88 and 89 (tomato, Hsinchu); PS90 (tomato, Kaohsiung); PS91 (sweet pepper, Kaohsiung); PS92 (potato, Taichung); PS94, 95 and 96 (eggplant, tomato and sweet pepper, respectively, Taipei); BP1, 2, 3, 4, 5, 6 and 8 (bird-of-paradise, Nantou), PS99 (eggplant, Changhua), and T145 (tobacco, Chiayi). Before being used in each experiment, the stock cultures were streaked on Kelman's TZC agar plates[8] and incubated for 48 hr at 30C. A single fludal colony from each strain was then transferred to YDC agar[4], incubated for 18-24 hr at 30C and used as the inoculum.

Bacterioncin production and detection The 52 strains of *P. solanacearum* were assayed on CPG agar medium[3] for bacteriocin production by the method of Vidaver et al[17] using all of the 52 strains as indicators. The surface of each CPG agar plate was spot-inoculated with 10 different test strains. The inoculated plates were incubated for 36 hr at 30C and used as master plates. Colonies on the master plates were then replica plated to fresh CPG plates and incubated further for 48 hr at 30C. The plates were inverted and exposed to vapor from 3 ml chloroform for 1 hr to kill the bacterial cells. Residual chloroform vapors were removed by placing the plates with lids off in a laminar flow for 30-60 min. Each plate was then overlaid with 4 ml molten (45C) CPG soft agar[3] containing 0.2 ml (10^7 colony forming units (cfu)/ml) of an indicator strain. After incubation for 24 hr at 30C, inhibition zones were observed and recorded.

Selection of avirulent strains from bacteriocin-producing strains Avirulent strains were selected from virulent strains of *P. solanacearum* showing broadspectrum of bacteriocin activity according to the procedure of Kelman and Hruschka[9]. The strains were cultured in TYG broth [9] for 5-10 days at room temperature. The bacterial cells were then removed from surface layer of the broth, streaked on the TZC plates, and incubated for 48 hr at 30C. Appearance of small butyrous colonies was considered as formed by avirulent cells[8]. A single butyrous colony was selected for each strain and purified by repeated platings on TZC medium. The aivurlent strains thus obtains were maintained in sterile distilled water in screw cap tubes at room temperature.

Pathogenicity tests were made on tomato seedlings grown in pots by stem-inoculation[7] with the avirulent strains to confirm their avirulence on tomato. The production of bacteriocin by the avirulent strains was also tested against

all indicator strains by the method described above.

Disease control experiments with avirulent bacteriocin-producing strains (ABPS) Effect of ABPS on bacterial wilt development was evaluated in a greenhouse[1]. Tomato (*Lycopersicon esculentum* Mill, "Shuang Hsi") plants were grown in clay pots containing sandy loam soil, one plant per pot. When the seedlings reached 15-18 cm high, they were removed from pots and roots were washed free of soils. Roots were then immersed in a water suspension of ABPS (10^9 cfu/ml) for 30 min and transplanted to 13-cm clay pots containing soils infested with 1.1×10^6 cfu/g dry soil of a virulent bacteriocin-sensitive strain PS68 of *P. solanacearum*. Roots similarly treated with sterile distilled water were served as controls. At 5-day intervals for 30 days after transplanting, the disease development was recorded using the disease index described previously[7]. Each treatment consisted of 5 plants and the experiment was repeated twice.

Multiplication of ABPS on tomato roots Tomato plants grown in pots in a greenhouse were removed and washed free of soils. Roots of the plants were dipped in a water suspension (2×10^9 cfu /ml) of ABPS (BP5A) for 30 min prior to transplanting in pots containing soils. The treated plants were returned to the greenhouse. Immediately and 5-day intervals for 30 days after treatment, three plants were removed for each determination. The roots were washed thoroughly with running water and rinsed with sterile distilled water thrice. The roots were blotted with tissue papers, excised, weighed and then ground with 10 ml sterile distilled water in motars. Samples (0.1 ml) of appropriate dilutions of the macerated suspensions were spread on a selective medium modified from that of Granada and Sequeira[5]. The modified medium contained TZC medium supplemented with 5 ppm crystal violet, 100 ppm polymyxin B sulfate, 20 ppm tyrothricin, 5 ppm chloromycetin, and 5 ppm cycloheximide. After 48 hr incubation at 30C, colonies were counted, and the number of colony forming units per g wet root was calculated. Multiplication of a virulent strain PS68 on the root was similarly determined.

RESULTS

Bacteriocin-producing strains The 52 strains of *P. solanacearum* tested were found to produce bacteriocins on CPG medium when all of the strains were used as indicator strains (Fig. 1). However, bacteriocins produced by different strains differed considerably in their spectrum of inhibitory activity; bacteriocins produced by some strains (e. g. PS21, PS51, PS67, PS68, PS70, PS71, PS91) were active against only one or a few indicator strains, while those produced by others (e. g. PS38, PS98, BP1, BP2, BP3, BP4, BP5, BP6, BP8) inhibited the growth of most of the indicators. All strains from bird-of-paradise (BP strains) were notably to have a broad spectrum of inhibitory activity and were selected for further study. To determine whether the inhibition zones observed on the plates were due to activity of phages, spot tests were carried out[14]. Agar blocks (0.5×0.5 cm^2 each) were picked out from the inhibition zones on CPG agar produced by the BP strains (BP2,

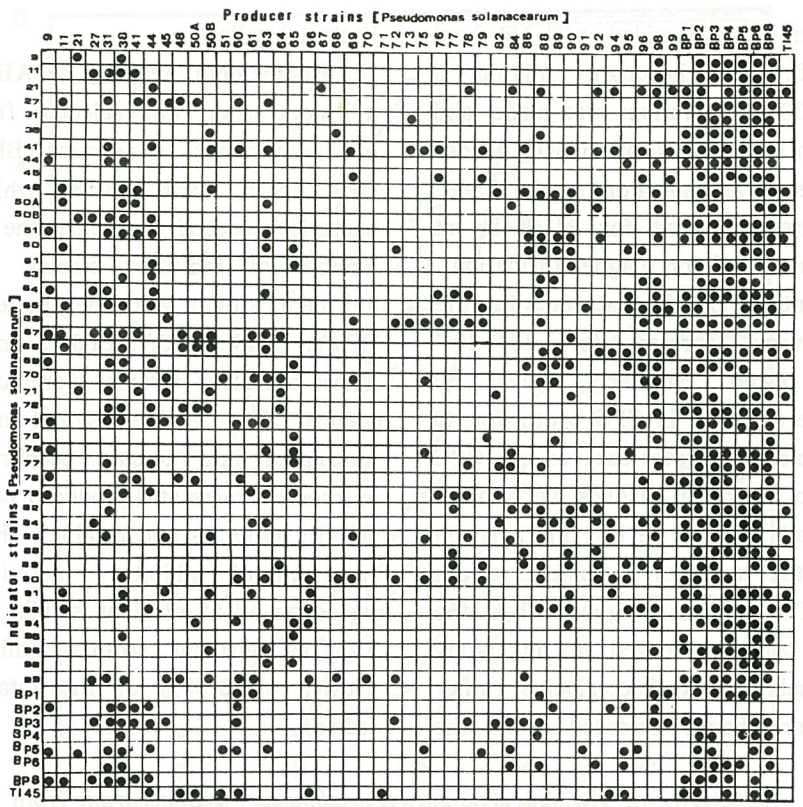

Fig. 1. Bacteriocins production by 52 strains of *Pseudomonas solancearum* on CPG agar medium using each of the 52 strains as an indicator. Squares with black dots indicate presence of inhibition zones, and squares without black dots indicate absence of inhibition zones.

Fig. 2. Inhibition zones on a lawn of an indicator strain PS68 of *Pseudomonas solanacearum* caused by bacteriocin(s) produced by virulent (BP5) and avirulent (BP5A) strains of the same bacterium.

4, 5, 6 and 8) and added each to 9 ml CPG broth, after thoroughly mixing, a series of 10-fold dilutions was made and spotted on CPG plates previously seeded with a sensitive indicator strain PS68. Plates were incubated for 24-48 hr at 30C and observed for plague formation. The results showed that no discrete plaques were formed for 10^{-1}-10^{-5} dilutions tested. Similarly, when BP strains were grown in CPG broth for 48 hr, and culture filtrates assayed on CPG plates, no individual plaques were observed for all 10-fold series dilutions on the indicator lawns of a sensitive strain PS68. The results suggest that the inhibition zones observed in this study were probably due to bacteriocins rather than bacteriophages released from the bacterial cells.

Bacteriocin activity of ABPS An avirulent strain was selected from each of six virulent BP strains (BP2, BP3, BP4, BP5, BP6, BP8) which have bacteriocin activity against the greatest number of indicator strains. The six avirulent strains were designated BP2A, BP3A, BP4A, BP5A, BP6A, and BP8A. These avirulent strains were unable to induce any visible symptoms when inoculated into tomato seedlings. The avirulent strains still produced bacteriocin activity (Fig. 2) and retained their wide spectrum of inhibitory activity when tested on all 52 virulent strains (Table 1). The avirulent strains inhibited the growth of 73-90% of the total strains tested.

Table 1. Sensitivity of virulent strains of *Pseudomonas solanacearum* from various hosts collected from Taiwan to avirulent bacteriocin-producing strains (ABPS) of the bacterium

ABPS[1]	No. of bacteriocin-sensitive strains isolated from							Percentage of total
	Tomato (28)[2]	Tobacco (5)	Potato (3)	Sweet pepper (5)	Eggplant (4)	Bird-of-paradise (7)	Total (52)	
BP2A	25	5	2	5	4	6	47	90%
BP3A	23	4	2	4	4	5	42	81%
BP4A	19	4	2	5	4	4	38	73%
BP5A	20	3	3	5	4	3	38	73%
BP6A	21	5	3	5	4	2	44	85%
BP8A	22	5	3	1	4	5	40	77%

1) The avirulent strains were selected from virulent strains of *P. solanacearum* isolated from bird-of-paradise.

2) Numbers in parentheses indicate the number of strains from each host tested for bacteriocin sensitivity.

Effect of ABPS on bacterial wilt development of tomato Symptom expression was delayed or disease severity was reduced on tomato plants treated with ABPS. The level of disease control, however, varied with ABPS (Fig. 3 and

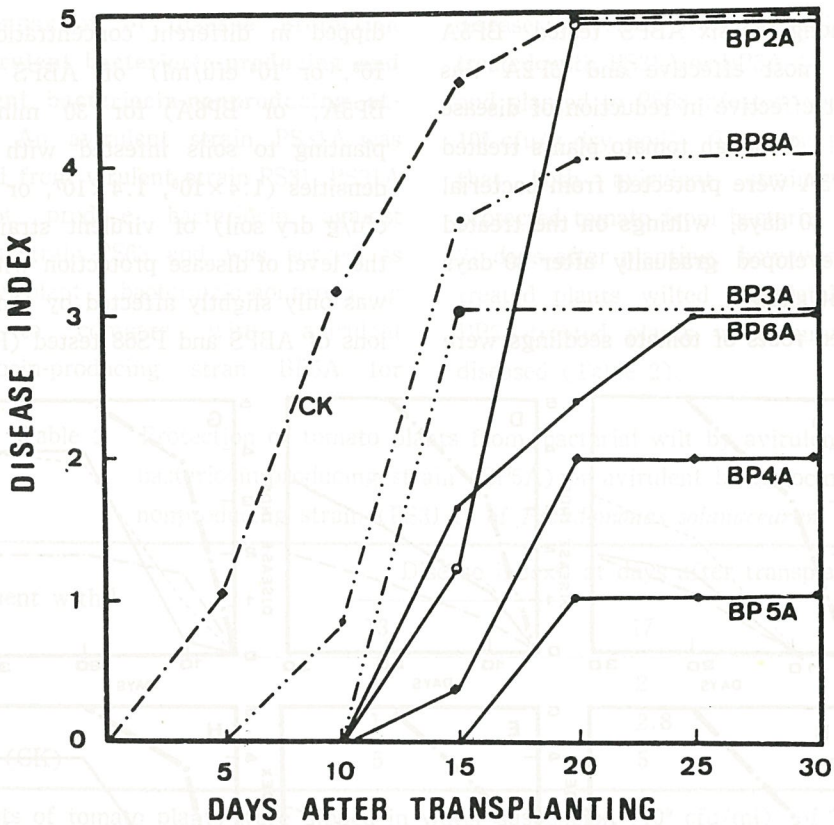

Fig. 3. Development of bacterial wilt of tomato plants treated with avirulent bacteriocin-producing strains (ABPS) of *Pseudomonas solanacearum*. Roots of tomato seedlings were dipped in a water suspension (10^9 cfu /ml) of ABPS (BP2A, BP3A, BP4A, BP5A, BP6A, or BP8A) or water (CK) for 30 min prior to transplanting in soil infested with virulent strain PS68 of *P. solanacearum* (1.1×10^6 cfu/g dry soil).

Fig. 4. Control of bacterial wilt of tomato by avirulent bacteriocin-producing strains (ABPS) of *Pseudomonas solanacearum*. Roots of tomato seedlings were immersed in ABPS (BP2A, BP3A, BP5A, BP6A or BP8A) suspension (10^9 cfu/ml) or water (CK) for 30 min before planting to soils infested with virulent strain PS68 (1.1×10^6 cfu/g dry soil) of the same bacterium.

4). Among the six ABPS tested, BP5A was the most effective and BP2A was the least effective in reduction of disease severity. Although tomato plants treated with BP5A were protected from bacterial wilt for 30 days, wiltings on the treated plants developed gradually after 30 days (data not shown).

When roots of tomato seedlings were dipped in different concentrations (10^7, 10^8, or 10^9 cfu/ml) of ABPS (BP4A, BP5A, or BP6A) for 30 min before planting to soils infested with various densities (1.4×10^6, 1.4×10^7, or 1.4×10^8 cfu/g dry soil) of virulent strain PS68, the level of disease protection with ABPS was only slightly affected by concentrations of ABPS and PS68 tested (Fig. 5).

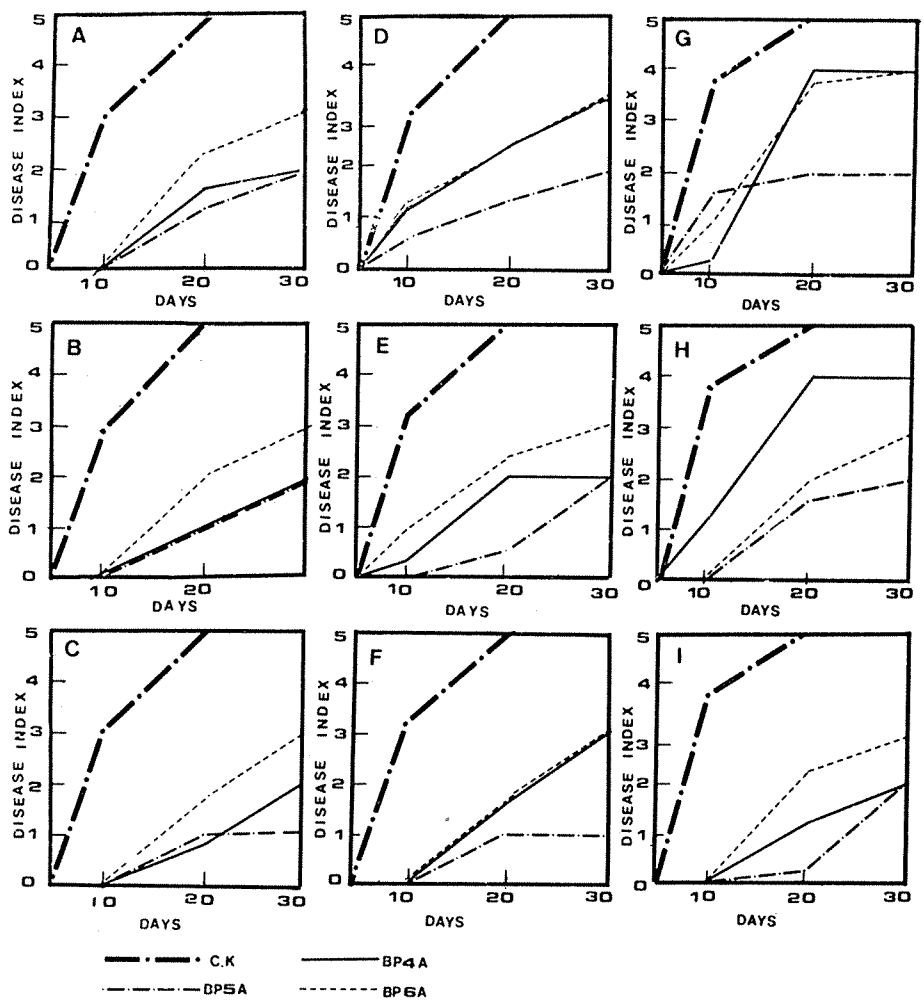

Fig. 5. Effect of concentration of avirulent bacteriocin-producing strains (ABPS) of *Pseudomonas solanacearum* on control of bacterial wilt of tomato. Roots of tomato seedings were dipped in 10^7 (A. D. G.), 10^8 (B. E. H.) or 10^9 cfu/ml (C. F. I.) of ABPS (BP4A, BP5A or BP6A) suspension or water (CK) prior to transplanting in soils infested with 1.4×10^6 (A, B, C), 1.4×10^7 (D. E. F.) or 1.4×10^8 cfu/g dry soil (G. H. I.) of virulent strain PS68 of *P. solanacearum*.

Comparison of disease protection by avirulent bacteriocin-producing and avirulent bacteriocin-nonproducing strains An avirulent strain PS31A was selected from virulent strain PS31. PS31A did not produce bacteriocin against virulent strain PS68 and was served as an avirulent bacteriocin-nonproducing strain to compare with avirulent bacteriocin-producing stran BP5A for disease protection. Tomato seedlings were treated with PS31A or BP5A (10^9 cfu/ml) and planted to PS68-infested soils (1.4×10^6 cfu/g dry soil). The result showed that both avirulent strains initially protected tomato from bacterial wilt. At 21 days after planting, however, PS31A-treated plants wilted completely, while BP5A-treated plants were only slightly diseased (Table 2).

Table 2. Protection of tomato plants from bacterial wilt by avirulent bacteriocin-producing strain (BP5A) or avirulent bacteriocin-nonproducing strain (PS31A) of *Pseudomonas solanacearum*

Treatment with[1)]	Disease index[2)] at days after transplanting		
	13	17	21
BP5A	1	2	2
PS31A	1	2.8	5
Water (CK)	5	5	5

1) Roots of tomato plants were dipped in water suspension (10^9 cfu/ml) of BP5A or PS3 1A, or in water only for 30 min prior to transplanting in soils infested with virulent strain PS68 of *P. solanacearum* (1.4×10^6 cfu/g dry soil).
2) Disease index: 0-5 (no symptoms-plant dead).

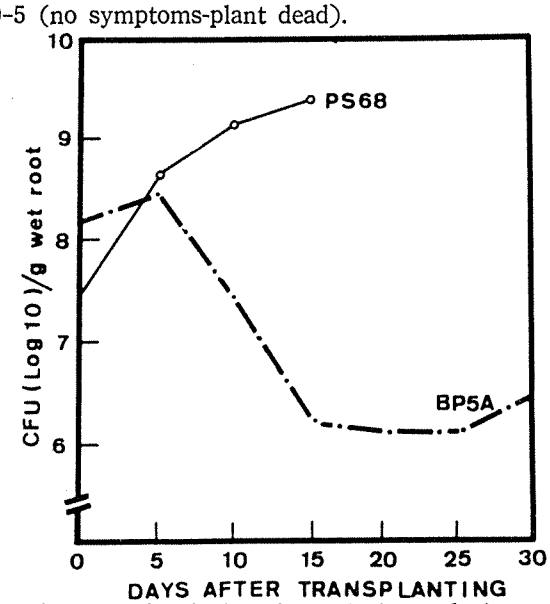

Fig. 6. Populations changes of avirulent bacteriocin-producing strain BP5A or virulent strain PS68 of *Pseudomonas solanacearum* on or in roots of tomato plants. Roots were dipped in suspension of BP5A (2×10^9 cfu/ml) or PS68 (1.3×10^9 cfu/ml) for 30 min before planting to the steam-treated soils.

Multiplication of BP5A and PS68 on roots Colonization of tomato roots by BP5A and PS68 is illustrated in Fig. 6. Population of BP5A slightly increased to 4.9×10^8 cfu/g wet root at 5 days after transplanting, then decreased sharply to 2.5×10^6 cfu/g wet root by 15 days, and remained more or less constant thereafter. For virulent strain PS68, the population increased gradually after planting and reached 3.5×10^9 cfu/g wet root at 15 days. Determination of the population was terminated after 15 days, because of the plants entirely wilted.

DISCUSSION

Bacteriocin production appears to be widespread in strains of *P. solanacearum*. Cuppels[2] reported that 48 strains of the bacterium from different hosts and regions of the world were bacteriocinogenic for one or more of the 53 indicator strains. Chen et al[1] tested 149 strains and found 121 strains had bacteriocin activity against 22 indicator strains. The results of the present investigation also revealed that 52 strains of *P. solanacearum* collected from Taiwan produced bacteriocins when all of the 52 strains were used as indicators. Bacteriocin-producing strains, however, differed greatly in their spectrum of inhibitory activity. In our study, notably, all strains from bird-of-paradise were found to have inhibitory activity against a greatest number of *P. solanacearum* strains. Sensitvity patterns suggest that different bacteriocins might be produced by *P. solanacearum*. However, a more detail study is required to reveal the nature of the bacteriocins produced. A nonsedimentable bacteriocin produced by avirulent *P. solanacearum* B1 has recently been isolated and characterized [3].

Avirulent bacteriocin-producing strains (ABPS) have been used or tested for control of bacterial plant diseases[16]. In this study, we demonstrated that preplanting treatment of tomato roots with ABPS derived from BP strains of *P. solanacearum* protected the plants from bacterial wilt. Similar results were also obtained by Chen et al in the control of bacterial wilt of tobacco with ABPS[1]. Therefore, ABPS appear to have the potential for use as biological control agents in control of bacterial wilt disease. The control mechanism was not determined in the present investigation. The data of the experiment on avirulent bacteriocin-nonproducing strain (PS31A) suggest that control is probably not due to a simple physical blocking of infection sites, since PS31A was not effective as ABPS (BP5A). However, not all ABPS were effective in disease control, even the most effective one, BP5A, did not protect tomato plants from bacterial wilt at later stages of tomato growth. The reasons for the different control efficacy observed among ABPS are not certain. There is possibility that some ABPS may not produce or produce insufficient amount of bacteriocins on the roots to combat the pathogen. Since effective biological control agents must be able to colonize the root systems, the difference in their colonization ability may also account for the different efficacy of ABPS to control the disease. Population assays indicated that BP5A did not multiply on the treated tomato roots. The inability to multiply and colonize the new growths of the root

system may be one of the reasons for the failure of protection by BP5A at later stages of plant development. Therefore, further selection of specific ABPS which can readily establish and multiply profusely on root systems is essential if effective and lasting control is to be achieved. The biocontrol agents should also produce sufficiently high concentrations of bacteriocins on the treated plants and are effective against most strains of the pathogen existing in the control areas concerned.

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Approaches to Biological Control of Soybean Root Diseases

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INTRODUCTION

Soilborne root-infecting fungi are among the most destructive pathogens of soybean. A diversity of such pathogens exists in Michigan, including *Pythium ultimum* (seed decay and seedling blight), *Phytophthora megasperma* f. sp. *glycinea* (root and stem rot), *Thielaviopsis basicola* (black root rot), *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium solani* (seed, seedling and root rots), *Macrophomina phaseolina* (charcoal root and stem rot), and *Phialophora gregatum* (brown stem rot, a vascular disease).

For many of these diseases, chemical control is not feasible for reasons of cost and insufficient efficacy. Moreover, varieties resistant to most of these diseases are not available and cultural practices are only partially effective. Therefore, it was considered worthwhile to investigate the possibilities of biological control as alternate means of dealing with some of these pathogens.

We have investigated the use of dried, green plant material as soil amendments for *T. basicola*; oilseed meals and urea as soil amendments for *T. basicola*, *P. ultimum* and *M. phaseolina*; and hyperparasites of the oospores of *P. megasperma* f. sp. *glycinea* for control of this pathogen, as a third approach. Some encouraging results have been obtained with all three approaches in laboratory and greenhouse experiments, but only those with urea have been verified in field experiments.

Control of *Thielaviopsis basicola* by germination-lysis.

Black root rot is a common disease of soybean in Michigan, especially during cool, spring periods when the root cortex can become badly decayed and the taproot frequently girdled. Papavizas[26] and Papavizas and Adams[27] studied the effects of organic amendments on black root rot of bean, and found that alfalfa hay stimulated germination of the chlamydospores of the pathogen in soil and that the germ tubes lysed without formation of new endoconidia or chlamydospores. We investigated this method for black

root rot of soybean and examined the mechanism of biocontrol in detail[31].

Alfalfa, red clover, rye and triticale were collected during flowering and were dried and ground (0.25 mm). All four plant residues, when mixed with soil at 1% (w/w), reduced chlamydospore populations of *T. basicola* to about 0.005 of the original population after 28 days. Disease was correspondingly reduced in soybean seedlings planted in treated soil 3-4 weeks after amendment. By contrast, chitin applied at 0.2% (w/w) reduced chlamydospore populations by only about 0.25, and was much less effective than the other amendments in reducing disease. In further studies with alfalfa, concentrations of 1% or 0.7% were more effective in reducing chlamydospore populations than 0.5%, which was more effective than a 0.1% concentration.

Higher alfalfa concentrations also stimulated greater germination of chlamydospores in soil than lower concentrations. Their germination on membrane filters in soil was stimulated during the first 1-2 days following incorporation of alfalfa. This was followed by lysis of the germ tubes, which was manifested by a decline in the number of chlamydospores with germ tubes, until at the 6th day no chlamydospores with germ tubes were seen. The stimulatory effect of the alfalfa amendment on germination was short-lived. Maximum germination took place when chlamydospores were applied to soil at the same time as the amendment. When chlamydospores were applied 1-3 days later, germination declined markedly and after 4 days no germination was seen. Chlamydospore populations showed a similar trend, i. e., the greatest decline occurred when chlamydospores and the amendment were applied simultaneously, whereas the application of chlamydospores 3 days after amendment resulted in only a slight decline in population.

The germination-lysis mechanism was confirmed by scanning electron microscopy. In alfalfa-amended soil, germ tubes emerged after 1-2 days. A few newly formed endoconidia, but no new chlamydospores, were seen. From the 3rd day on, hyphae collapsed and gradually lysed apparently by autolysis, since colonization of the hyphae by other microorganisms was not seen. Chlamydospores recovered after long periods were empty and badly eroded. In nonamended soil, the chlamydospores remained ungerminated and intact.

Studies were done to determine which components of the alfalfa hay were responsible for germination stimulation. Unsaturated fatty acids had previously been implicated as the stimulatory substances[1,28], based on germination stimulation in soil by individual fatty acids, triglycerides and vitamin E acetate, but not by single simple sugars, amino acids and sterols[28]. However, in our work[31], aqueous extracts of alfalfa were more stimulatory in soil than extracts made with the fat solvents diethyl ether or chloroform-methanol. Neutral, cationic and anionic fractions of the aqueous extracts all were stimulatory, but less so than the combined fractions or the original aqueous extract. Chlamydospore populations in soil were reduced much more by aqueous extracts than by organic solvent extracts of alfalfa.

It seems clear that biological control was achieved through stimulation of

germination of chlamydospores, mainly by a mixture of water-soluble components of alfalfa hay, and that this was followed by autolysis of hyphae and degradation of the chlamydospores, resulting in a precipitous population decline. A secondary mechanism might also be involved: the fungistatic capacity of soil was elevated following application of alfalfa hay. Alfalfa (1%) was applied to soil, then 9 days later chlamydospores were applied simultaneously with a second application of alfalfa (1%) to stimulate germination. Germination in soil which had received the first amendment was 55%, whereas that in soil which received only the second amendment was 79%. Adams and Papavizas[1] also showed that treatment of soil with alfalfa hay increased fungistasis against *T. basicola*. Thus, germination in rhizospheres (and hence numbers of root infections) would be expected to be less in soil treated with green plant residues, and in fact chlamydospores and endoconidia of *T. basicola* adjacent to germinating bean seeds failed to germinate in soil treated 7 days earlier with alfalfa hay, whereas an appreciable number germinated in soil not so treated[1].

The destruction of inoculum by the germination-lysis method of biological control is conceptually an attractive one, and should be persistent since inoculum density is reduced. Populations of a number of other pathogens have been reduced by this same mechanism, but the method is not generally applicable to all [30]. However, the method is difficult to apply in practice due to a requirement for a large amount of organic material (ca. 1% by weight), the probable necessity for a homogeneous mixture of the residue with the soil, and a waiting period of 3-4 weeks for phytotoxicity to disappear. It was suggested that a winter cover crop of rye might be plowed down in the spring several weeks before seeding soybeans[31]. This was attempted in small field plots, but dry, hardened soil prevented deep and thorough mixing of the plant material with the soil. Even so, populations of *T. basicola* was reduced by about 50% (J. L. Lockwood and J. E. Bailey, unpublished results).

There may be alternate means of stimulating germination of propagules in soil, possibly by specific chemicals, particularly volatile compounds which can permeate the soil. Brief exposure of soil to distillates of alfalfa hay containing methanol, isobutyraldehyde and isovaleraldehyde stimulated germination of microconidia of *Verticillium albo-atrum*, followed by a precipitous decline in population[18]. Such materials might be effective in concentrations too low to support growth, and hence might have broader applicability among fungi. For example, certain fatty acids and their volatile peroxidation products stimulated germination of conidia of *Alternaria alternata* and *Fusarium solani* f. sp. *pisi* in soil at extremely low concentrations [19]. The most stimulatory compound, 2,4-hexadienal, stimulated germination at 4 nL/petri dish. The stimulation of germination of propagules with low concentrations of volatile compounds which cannot act as substrates for colonization would appear to be an attractive area for further study.

Control of several pathogens by oilseed meals and urea.

Experiment with oilseed meals. *Fusarium oxysporum* and *F. solani* are involved in soybean seedling and root rots, and their response to several orga nic amendments in soil was investigated. Later, *P. ultimum* also was included.

Ten different kinds of residues were evaluated: ground mature stems of barley, wheat, corn and soybean; commercial sugarbeet pulp; alfalfa meal; linseed, cottonseed and soybean meals; and ground crabshell[36]. When applied at 1% (w/w) to a sandy loam soil in closed containers, only the oilseed meals and chitin reduced *Fusarium* populations, after 4 weeks. The oilseed meals were most effective, reducing populations to 0.001 to 0.0001 of the original population. Therefore, these products were studied further.

The three oilseed meals were effective at concentrations as low as 0.25%[36]. Composting the oilseed meals and alfalfa meal neither increased nor decreased their effectiveness. Populations of actinomycetes, bacteria and fungi were reduced in soil treated with oilseed meals, but fungi were reduced the most. Soybean meal reduced populations of *Fusarium* to a similar extent in soils adjusted to -3.0, -0.2, -0.03 and 0 bars matric potential, but cottonseed and linseed meals were most effective at -0.2 and -0.03 bars.

The oilseed meals were less effective in fine-textured than in coarse-textured soils[36]. For example, *Fusarium* populations in Capac loam (2.96% organic matter) were reduced less than those in Oshtemo-Boyer sandy loam (2.02% organic matter), and populations in Brookston loam (6.46% organic matter) were not reduced at all, even after 10 weeks.

In view of the encouraging results in the laboratory, two field experiments were done in a loamy sand soil using linseed and cottonseed meals at 1% concentration in uncovered plots[36]. However, after 4-5 weeks *Fusarium* populations were equivalent to or higher than those in the untreated plots; after 9-12 weeks, *Fusarium* populations in amended plots were higher than those in control plots.

The discrepancy between laboratory and field results led to studies of the mechanism of action of the oilseed meal amendments in the hope of improving field performance[37]. The greater reduction of *Fusarium* populations in oilseed meal-supplemented soil in closed than in open containers, and the failure of oilseed meals to reduce *Fusarium* populations in field experiments done in uncovered plots, suggested the involvement of volatile degradation products of the meals. This was confirmed by the near extinction of *Fusarium* in small soil samples contained in planchets incubated on the surface of, but separated from contact with, a larger mass of soil to which 1% oilseed meals had been added. Further, alkaline volatiles were trapped in small beakers of distilled water or 2% boric acid solution placed in the containers. Soil treated with soybean meal yielded the greatest amount of titratable alkalinity of the three oilseed meals, in boric acid traps. The presence of trap solutions nullified the toxic effect of soybean meal, but not that of linseed and cottonseed meals, suggesting a more complex array of volatile toxic substances in soil containing these amendments.

The trapping of alkaline volatiles in boric acid suggested the presence of ammonia and this was confirmed by gas chromatography of the boric acid trap solutions[37]. Ammonia was first detected in 2-3 days, then increased rapidly in concentration until about the fifth day when the rate of production declined. Ammonia production was in the order: soybean meal linseed meal cottonseed meal. Peak ammonia concentrations were 3747, 2187 and 1394 μg/ml of trapping solution, respectively, for the three amendments. By contrast, the ammonia concentration in nonamended soil or alfalfa-amended soil was 10 μg/ml. Amines were not detected. The C/N ratios of the oilseed meals were 6.7-7.4 as compared with 17.1 for alfalfa meal, apparently accounting for the production of ammonia by the oilseed meals.

The behavior of *Fusarium* chlamydospores in oilseed meal-amended soils was studied microscopically. When chlamydospores were introduced into soil simultaneously with the amendments, 95% or more germinated after 24 hours, whereas none germinated in nonamended soil. After 2-3 days, hyphae lysed and new chlamydospores were produced. In subsequent observations, these new chlamydospores failed to germinate in soil and all hyphae disappeared. The chlamydospores progressively lost viability as shown by tests on agar, until at 21 days none remained germinable, whereas in nonamended or alfalfa-amended soil, 65% were still germinable after 21 days. When chlamydospores were added to soil 7 days after amendment, their ability to germinate when transferred to agar 7 days later was near zero, whereas those incubated for 7 days in nonamended or alfalfa-amended soil germinated 80% or more.

The lethal effect of volatiles from oilseed meals was not restricted to *F. oxysporum* and *F. solani*. Chlamydospores of *F. lateritium*, *F. moniliforme*, *F. rigidisculum*, *F. roseum*, and *F. tricinctum* also where decimated when incubated in soil planchets placed on soil amended with oilseed meals. Incubation in the presence of nonamended or alfalfa-amended soils had no effect.

It is interesting that the oilseed meals initially nullified soil fungistasis, promoting germination of *Fusarium* chlamydospores. This was followed by growth of hyphae and production of new chlamydospores, which were killed, apparently by the ammonia and possibly other toxic volatiles generated during decomposition of the meals. Thus. the mechanism of biological control of *Fusarium* by the oilseed meals differs from that for *T. basicola* by dried, green plant material. In the latter case, the fungus was eradicated after germination because of autolysis, presumably from nutrient stress[24], and because this fungus, unlike *Fusarium* spp., was unable to produce new chlamydospores.

Subsequent to the above work, soybean meal was shown to reduce populations of *P. ultimum* to undetectable levels in covered containers of soil[5,14]. In open containers, the population first increased, then declined to a lower level over time. In field experiments with 1% soybean meal in covered and uncovered plots, populations of *P. ultimum* increased, sometimes many-fold, then declined, but frequently to levels no less than those

of the original population[14]. The ineffectiveness of the oilseed meals against *Fusarium*[35] and *P. ultimum*[14] in field conditions was due apparently to their being colonized by the pathogens before lethal concentrations of ammonia were generated. For reasons as yet unknown, the ammonia subsequently formed did not bring about control.

Experiments with urea. To circumvent the problem of colonization of the amendment by the pathogen, urea was evaluated as an alternative source of ammonia, since it does not serve as an energy substrate. In laboratory experiments, urea at 1% concentration in soil reduced the population of *P. ultimum* without an initial increase[5]. Therefore, urea was tested for its effect on populations of *P. ultimum*, *M. phaseolina* and *T. basicola* in a sandy loam soil in the field[7]. The soil was artificially infested with the three pathogens and packed into clay tiles sunk into the surrounding field soil. After an equilibration period, this soil was watered with an aqueous solution of urea to give 0.1 and 1% (w/w) urea in trials 1 and 2 and 0.25, 0.5 and 1% urea in trial 3.

Populations in untreated soil remained stable throughout the 30-40 days of each experiment. In soil treated with 1% urea, populations of all three pathogens were either reduced to extinction or to very low levels. Their relative sensitivity was in the order: *P. ultimum T. basicola M. phaseolina*. Urea at 0.25 and 0.5% also drastically reduced populations, but the rate of reduction was slower at these concentrations. 0.1% urea reduced populations in trial 1 but not in trial 2. Trial 2 was done in the autumn when soil temperatures were frequently 10 C, possibly retarding the rate of formation of ammonia or reducing its toxicity. These results are consistent with those of Papavizas *et al.*[29] that bean root rot caused by *T. basicola* was reduced in soil amended with soybean meal at 35, 28 and 22°C, but not at 16°C. Urea treatments of soil were accompanied by an increase in soil pH, depending on the concentrations applied; with 1% urea, pH rose to 8.7-9.0 after 4 days, then declined to about pH 8.0 at the end of the experiment. With 0.25% urea, pH rose to 8.0, then declined to the original value of 5.7 before the end of the experiment.

Ammonia concentrations in soil with 1% urea were about 500 mg/kg soil throughout the experiments; with 0.25% urea, ammonia reached a peak of about 300 mg/kg at 4 days, then declined to about 100 mg/kg.

Thus, what began as a biological control approach to root disease control through the use of plant materials as soil amendments ended as a chemical control approach. The practical utility of the application of a high concentration of nitrogenous material to agricultural soils is questionable. Even at 0.25%, the equivalent application of about 5000 kg urea/hectare is 6-8 times the normal high rates for fertilizing wheat and corn. Excess nitrogen could be detrimental to crops and would be expensive. However, further research might focus on urea applications to localized foci of infection, or the use of lower concentrations, which might bring about a gradual population decline.

Effect of ammonia on exudation from fungal propagules. Since the

decline in pathogen populations in soil proceeded much more slowly at low than at high concentrations of urea, it seemed possible that this slow decline might be related to gradual attrition of the propagules than to a direct lethal effect. Exudation of ^{14}C -labelled compounds from fungal propagules is increased in soils and has been associated with the imposition of soil fungistasis[15,16,24]. Prolonged incubation of fungal propagules in soil can debilitate them, resulting in elevation of their nutrient requirements for germination[4,16], in progressive loss of viability[4,16], and in decreased virulence[2,13]. These aspects of fungal behavior in soil can be mimicked by a model apparatus in which fungal propagules are incubated on a bed of sand through which a dilute salts solution is percolated, thereby steepening the diffusion gradient away from the propagules in a manner analogous to that brought about by microbial competition for substrate in soil. Therefore, studies were done to determine whether ammonia might increase exudation from fungal propagules *in vitro* and in soil, and to determine the effects of the increased exudation on propagule viability.

Exposure of conidia of *Drechslera victoriae* and sclerotia of *M. phaseolina* for a few hours to high free ammonia concentrations (up to 10,000 mg/L) in the model system stimulated a short burst of exudation, which was associated with death of the propagules[6]. Lower concentrations increased exudation less, but the increased exudation tended to be maintained for a longer period. Viability of the propagules was reduced when they were continually exposed to doses that were nonlethal for shorter durations. High pH by itself also increased exudation somewhat, but did not account for the greater exudation effected by ammonia, nor for its lethal effects. In another model system imposing a lesser diffusion stress, ammonia at low concentrations (50 mg/L) actually decreased exudation and viability was not affected. However, as ammonia concentration was increased (50 mg/L), exudation was stimulated and viability declined[6]. The decreased exudation and lack of toxicity of low concentrations of ammonia also were observed by Loffler and Schippers[25] for conidia of *F. solani* f. sp. *phaseoli*, *D. victoriae* and *Botrytis cinerea*.

Since concentrations of ammonia from treatments of soil with 0.25% urea exceeded 50 mg/L, it is possible that low concentrations of ammonia can accelerate the natural attrition of propagules occurring under nutrient stress in soil, through increased exudation depleting endogenous reserves.

Biologica control of Phytophthora megasperma f. sp. glycinea by hyperparasites of the oospores

Laboratory experiments. During observation of the behavior of *P. megasperma* f. sp. *sojae* (*Pmg*) in soil, numerous oospores of the pathogen were found to contain fungal, actinomycete, and less frequently bacterial parasites[32]. Only a few hyperparasites of oospores in soil had been described[9–12,20], and therefore it was surprising to find a wide range of microorganisms parasitizing oospores of *Pmg*. The hyperparasites found initially included the Oomycetes *Pythium monospermum* and *Leptolegnia*

sp., the Hyphomycetes *Dactylella spermatophaga*, *Diheterospora chlamydosporia*, *Humicola fuscoatra*, *Fusarium oxysporum*, *Cephalosporium* sp. and *Alternaria alternata*; the Actinomycete, *Actinoplanes missouriensis*; three Hyphochytridiales, *Rhizodiomycopsis japonicus*, *Canteriomyces stigeoclonii*, and *Hyphochytrium catenoides*; and the bacterium *Pseudomonas* sp. Subsequently[35], six additional Actinomycetes isolated from sources other than fungal oospores were found to be efficient parasites of oospores *in vitro*. These included *Actinoplanes philippinensis*, *A. utahensis*, *Amorphosporangium auranticolor*, *Ampullariella regularis*, *Spirillospora albida* and *Micromonospora* sp.

Oospores were parasitized most frequently by Oomycetes, Hyphochytridiomycetes and *A. missouriensis* in wet soils and by Hyphomycetes in drier soils, suggesting that different groups are adapted for hyperparasitic activity under different soil moisture conditions[32].

The ability of several hyperparasites to parasitize oospores was compared by incubating oospores of *Pmg* on membrane filters on sterile soil that had been infested individually with several hyperparasites[22]. *H. fuscoatra*, *H. catenoides* and *P. monospermum* were the most efficient parasites, infecting at least 76% of the oospores within three weeks. *H. catenoides* and *P. monospermum* reached high levels of parasitism within 1 week; *H. fuscoatra* showed no parasitism during the first 2 weeks, but by the third week nearly 90% of the oospores were parastized. Parasitism by *Cephalosporium* sp., *D. spermatophaga*, *D. chlamydosporia* and *F. oxysporum* was 25% or less. These same seven fungi did not parasitize mycelium of *Pmg* in paired cultures on cornmeal agar or PDA, nor were there inhibition zones or any other evidence of interaction[22].

Some environmental parameters for parasitism by specific hyperparasites were studied in steamed soil. Parasitism by *H. catenoides* in flooded soil during 10 days did not occur at 16°C, but was substantial (60%) at 20°C and maximum (85%) at 28°C[22]. *P. monospermum* parasitized 32% of the oospores at 16°C, reached a maximum of 67% at 24°C, then sharply declined to parasitize 20% at 28°C. Parasitism by *H. fuscoatra* after 7 days at -0.3 bar matric potential was very low at 5-15°C, but was progressively greater at 20, 25, and 30°C, with 92% of oospores parasitized at 30°C[35]. Results for *A. missouriensis* at 0 bars were similar, except that parasitism at 15°C was quite high (63%). Parasitism by *A. missouriensis* at constant 25°C was maximum (85%) at 0 bars matric potential and decreased with decreasing matric potential to 59% at -15 bars[35]. The frequency of oospore parasitism by *H. fuscoatra* was 62% at 0 bars; it increased to 91% at -0.3 bars, then decreased to 67% at -15 bars. Thus, those parasites so far tested were active over a fairly broad range of moisture and temperature conditions.

The oospore hyperparasites appear to be quite nonspecific with respect to their oomycete host range. Oospores of *Pmg*, *Phytophthora cactorum*, *Pythium* sp. and *Aphanomyces euteiches*, all were parasitized by Hyphochytridiomycetes in flooded soils[32], but in such a test infection could have been by different organisms. In tests on water agar,

isolates of *A. missouriensis*, *H. fuscoatra*, *H. catenoides*, *P. monospermum* and *V. chlamydoporia* were tested in all possible combinations against oospores of *Pmg*, *P. aphanidermatum*, *P. ultimum*, *A. cochlioides*, *A. euteiches* and *Phytophthora citrophthora*[35]. After two weeks all host species were parasitized by all parasites except *P. ultimum* by *H. catenoides*. Parasitism ranged from a low of 14% to a high of 96% (3 instances), and in 18 of the 30 combinations, in excess of 50% of oospores were parasitized. *A. missouriensis*, *H. fuscoatra* and *V. chlamydosporia* were the most efficient of the five parasites overall. Although *H. catenoides* did not parasitize *P. ultimum* in our experiments, it did so in those of Ayers and Lumsden[3].

The actinomycetes *A. philippiensis*, *A. utahensis*, *A. auranticolor*, *A. regularis*, *S. albida* and *Micromonospora* sp., each parasitized 94-99% of *Pmg* oospores *in vitro*[35]. These organisms were not known previously to be oospore parasites.

Oospores from cultures 16, 22, or 30 days old were infected equally by *A. missouriensis* and *H. fuscoatra*. Sporangia of *A. missouriensis* from cultures 13, 29 or 45 days old and aleurospores of *H. fuscoatra* from cultures 12, 28 or 78 days old, all parasitized 95% of *Pmg* oospores[35]. Thus, within these limits, oospore susceptibility and hyperparasitic capacity were unaffected by age.

A question can be raised whether oospores produced in culture were similar in susceptibility to parasitism to those produced in natural substrates. Thus, oospores produced during 5 weeks in V-8 juice broth were compared with those produced during 5 weeks in aseptically grown soybean seedlings[22]. Oospores from the two sources did not differ greatly in susceptibility to parasitism when incubated in six different soils for 1 or 2 weeks. In flooded soils there was no difference between the two types of oospores; in drier soils there was more infection of culture-grown than of seedling-grown oospores in four of 12 comparisons, but no difference in the other eight comparisons.

There is also a question whether the oospores were viable. With respect to *Pmg*, other work in our laboratory frequently showed germinability as great as 50%[23]. Thus, viability was at least this great and probably greater, since virtually all of the oospores contained apparently living protoplasm, and as many as 65-70% assumed the "activated" stage. Since the incidence of parasitism often exceeded 90%, living oospores must have been parasitized. Another test of oospore viability is staining with a vital stain. Over 85% of *Pmg* oospores from various sources were stained with tetrazolium bromide (MTT)[34]. Hence, it appears that a high proportion of the oospores used in experiments with hyperparasites were viable.

There appears to be little specificity of parasitism with respect to race of *Pmg*. Humble and Lockwood[22] found that 60-65% as many oospores of race 7, as compared with races 1 and 3, were parasitized by *H. catenoides* and *P. monospermum*, but Sutherland and Lockwood[35] found more than 95% of the oospores of races 1, 3, 4 and 7 parasitized by *A. missouriensis* or *H. fuscoatra*.

The invasion of oospores of *Pmg* by

A. missouriensis and *H. fuscoatra* was compared by transmission electron microscopy. Both organisms rapidly penetrated the oogonial and oospore walls without production of specialized structures and with no evidence of enzymatic degradation of the walls. No apparent morphological or internal structural changes occurred in response to hyperparasitic invasion. Thus, infection by a prokaryote and eukaryote appeared to be similar.

Biological control experiments. Although hyperparasitism of oospores introduced into natural soils occurs frequently[22,23], whether it is of biological significance in reducing populations of oospores is unknown. In one approach to this question, two soil samples from an apple orchard, one where trees were infected with *P. cactorum* and the other where trees were not infected, were incubated with oospores of *P. cactorum*, *Pmg*, *Pythium* sp. and *A. euteiches*. Oospore infection of all four species was significantly greater in soil collected near healthy trees than in soil near trees infected with *P. cactorum* (e. g., 58% vs. 24%, respectively, for *P. cactorum*), suggesting a possible relationship of a high incidence of parasitism to pathogen suppression.

An attempt was made to determine if such a relationship existed in soils of different potentials for Phytophthora root rot of soybean[22]. Soils from 33 soybean fields were assayed for Phytophthora root rot potential by incubating soybean seedlings in flooded soil samples. Fifteen soils were selected, five giving severe root rot, five moderate root rot and five no disease. Oospores of *Pmg* were incubated for 1, 2, and 3 weeks in soils kept either flooded or at a lower water potential. However, there was no significant relationship between the percentages of oospores parasitized and the disease potentials of the soil at either moisture condition. In flooded soils, in which parasites could be identified, parasitism was mainly by Actinoplanaceae or Hyphochytridiales, but the root rot potentials of soils could not be correlated with particular parasite distribution patterns. It is possible that our methods were not sufficiently precise to demonstrate a correlation between hyperparasite activity and disease potential. Possibly, an incubation period longer than 3 weeks may be necessary to allow full expression of hyperparasitic activity.

Despite our finding no evidence for a role for hyperparasitism in suppressing Phytophthora root rot of soybean in field soils, the possibility that an augmented population might effect biological control was considered worthy of testing.

An essential character of biological control agents is that they not be pathogenic themselves to the plant species they are being used to protect. Soybeans were planted in steamed soil that had been infested with *D. spermatophaga*, *D. chlamydosporia*, *H. catenoides*, *H. fuscoatra* and *Cephalosporium* sp[22]. After 5 weeks, the emergence, survival, and dry weights of plants grown in the infested soils did not differ significantly from control plants. In addition, roots and foliage of treated and control plants were comparable in color, length and general vigor. Thus, there was no evidence for pathogenicity of the hyperparasits to soybeans.

In preliminary biocontrol experiments

in the laboratory, *H. fuscoatra*, *H. catenoides*, *P. monospermum* and a combination of *H. catenoides* and *P. monospermum* were incubated for 7 days in soil naturally infested with *Pmg*, then soybeans seedlings were applied[35]. *H. catenoides* and *P. monosporum* or the combination of the two reduced severity of disease in two of three experiments; *H. fuscoatra* reduced disease in one experiment. This led to a number of biocontrol experiments in the greenhouse.

Mycelia of *H. catenoids* applied on a vermiculite carrier to soil artificially or naturally infested with *Pmg*, afforded a high degree of protection against Phythophthora root rot[21]. Seedling emergence, plant height and root health frequently approached those of plants grown without the pathogen, but disease was never completely eliminated. Control was similar whether soil was treated with *H. catenoides*, then flooded for 7 days before seeding, or if the flooding and prior incubation periods were eliminated.

In other experiments, hyperparasites were applied as coatings on soybean seeds. In experiments done in a greenhouse mix artifically infested with *Pmg* oospores, seeds coated with aqueous suspensions of *A. missouriensis* or *H. fuscoatra* gave plant stands 1.4- to 4-fold higher than those from noncoated seeds [35]. Roots of plants from coated seeds appeared to be healthy. Biocontrol was achieved whether oospores were produced in V-8 juice broth or in soybean tissue.

In further work, soybean seeds were coated with the actinomycetes *A. missouriensis*, *A. utahensis*, *Micromonospora* sp. or *A. auranticolor*, or the fungus *H. catenoides*[17]. The organisms were grown in yeast extract-starch broth for 4-6 weeks, then comminuted, washed and applied to soybean seeds in a carrier of 1% carboxymethyl cellulose + 1% soluble starch in water. Seeds were sown in a loam soil either naturally infested with *Pmg* or supplemented with 400 oospores of *Pmg*/*g*.

Stands of soybeans from seeds coated with *A. missouriensis*, *A. utahensis* or *Micromonospora* sp. were increased over those from noncoated seed, in three of four experiments; *A. auranticolor* was effective in one experiment. In the experiment giving maximum control, stands were increased 2- to 2.6-fold and in some instances to levels not significantly different from healthy control plants in steamed soil. Treasments giving increased stands also tended to have reduced root rot severity and the surviving plants frequently had increased root and shoot weights, as compared with plants from nontreated seeds. *H. catenoides* was not effecive as a seed treatment, which was surprising since it gave excellent control when appled to soil in other experiments[21].

The disease control achieved by seed treatments[17] and by application of *H. catenoides* inoculum to soil at the same time as oospore inoculum[21] raises a question about the mode of action of the hyperparasites. In such experiments, can they parasitize oospores rapidly enough to account for disease control, or are they acting by some other means. The question cannot now be answered with certainty, since there is no precise means of quantifying changes in the inoculum density of *Pmg*. An approach to this

question was made using soybean seedlings as baits for *Pmg* in hyperparasite-treated soil. Soil was incubated for 1 and 3 weeks after which 10- fold dilutions were made and soybean seedlings applied. Soil treated with *A. utahensis* or *A. missouriensis*, in one experiment, gave less seedling disease than control soil at the greater soil dilutions. In a second experiment, all five hyperparasites gave less disease than control seedlings at the greater soil dilutions. Though this approach provides evidence for reduction in oospore populations, it still does not say how rapidly they can act. Electron microscopic studies of parasitized oospores *in vitro* showed extensive invasion of the oospores by *A. missouriensis* and *H. fuscoatra* after only 4 days of incubation [33], but earlier examinations were not made.

Relatively little has been done to evaluate different media for the production of inoculum of hyperparasites or the longevity of harvested inoculum in storage. Selection of appropriate growth media is especially important for the Hyphochytridiales and Actinoplanaceae, since these organisms grow slowly. Of several different media evaluated for prodution of *H. catenoides*, tryptone-glucose-yeast extract agar (TGYE) [21] supported the most growth, and growth was further enhanced when the medium was prepared with 0.2% agar to make semi-solid medium in which the fungus grew suspended. Mycelia of *H. catenoides* from TGYE cultures mixed with sterile vermiculite and stored at 25°C in plastic bags yielded about 60% of the original number of colonies after 6 months. Filonow and Lockwood[17] obtained satisfactory growth of several Actinoplanaceae and *H. catenoides* in Emerson's yeast extract-starch broth.

Hyperparasites have demonstrated potential for biological control of Phytophthora root rot of soybean in laboratory and greenhouse experiments. These results, the great diversity and broad host ranges of the hyperparasites, and the fairly wide range of soil temperatures and moistures over which parasitism occurs lend encouragement to further work on this group of biocontrol agents, not only for control of Phytophthora root rot of soybean, but for other oomycetous root diseases as well. Seed coating would seem to be the method of choice in further studies. It has given satisfactory results in greenhouse tests and would likely be a more economical and acceptable practice than broadcast application or incorporation with the seed. Further research should focus on such factors as strain selection, concentration and form of inoculum, carriers of inoculum, culture media for inoculum production, and inoculum storage potential. Field evaluations of efficacy also need to be made. Attempts so far have been frustrated because of inability to obtain sufficient disease[35].

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